Development of lipid-based enteric coatings

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Gutachter:

1. Prof. Dr. rer. nat. habil. Karsten Mäder

2. Prof. Dr. rer. nat. Michaela Schulz-Siegmund

3. Prof. Dr. Lea Ann Dailey

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“I have learned that success is to be measured not so much by the position that one has reached in life as by the obstacles which he has overcome while trying to succeed.”

"Nauczyłem się, że sukces powinien być mierzony nie tyle pozycją, jaką się osiągnęło w życiu, ile przeszkodami, jakie się pokonało w dążeniu do sukcesu".

Booker T. Washington (1856-1915)

Dedicated to my family

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1. Introduction

The aim of the current work was to develop enteric coatings which had to consist only of substances approved for use in food products and nutraceuticals. As the principal coating material, determining the release of an active substance from a dosage form lipids were chosen. The selected lipids are widely ingested with everyday food. They are classified as GRAS (generally recognized as safe) substances. Lipids, due to their attributes and fate in the human body, could potentially be used for the formation of pH-sensitive enteric coatings (e.g. in gastro-resistant dosage forms). The development of pH-sensitive enteric coatings consisting of substances permitted for use in foods will be a major achievement. These coatings will be willingly applied in phytopharmaceuticals, dietary supplements, food products and nutraceuticals where the use of food grade substances is of great importance. So far, no effective pH-sensitive coating has been developed that would employ nontoxic, biodegradable substances and simultaneously ensure a reliable enteric drug delivery.

Enteric coatings applied to oral solid dosage forms may offer several functions. These coatings prevent the release of active substances in the stomach and allow drug release in the intestine [1,2]. The reasons for using enteric coatings are as follows: 1) the prevention of the actives degradation in the gastric environment (e.g. erythromycin, digoxin, omeprazole); 2) the protection of stomach mucosa from irritations causing by active substances (e.g. salicylic acid derivatives, sulfonamides, iron preparations) ; 3) the delivery of API(s) to specific sites in the intestine (for the treatment of local diseases, for a better absorption); 4) the avoidance of interactions of certain drugs with pepsin and peptones that could lead to a hindrance of gastric digestion [3–6]. In pharmaceutics, enteric coatings are based on pH-sensitive polymers [3]. These polymers act by utilizing pH differences existing in the gastrointestinal tract [7,8]. The pH-sensitive polymers contain carboxylic groups, which remain unionized in the stomach. The ionization of carboxylic groups occurs at higher pH values in the intestine allowing the dissolution of the coating and the drug release [9]. The most common polymers used in pharmaceutical dosage forms to provide enteric drug delivery include: cellulose-based polymers (cellulose acetate phthalate, hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate), polyvinyl derivatives (polyvinyl acetate phthalate), anionic polymethacrylates (poly(methacrylic acid, methyl methacrylate), poly(methacrylic acid, ethyl acrylate)) [4,6,10–13]. These polymers are synthetically or semi-synthetically obtained and they are not approved for use in food products [14]. A naturally occurring polymer which can also be applied in enteric coatings is shellac. Shellac is a nontoxic, biodegradable, physiologically harmless substance listed as GRAS (Generally Recognized As Safe) by Food And Drug Administration (FDA) [15]. It is obtained from the resinous secretion of the insect *Kerria lacca* [16]. The major components of shellac are aleuritic acid, shellolic acid and jalaric acid, as well as butolic and kerrolic acids [13,17].

Shellac is practically insoluble in acidic and pH-neutral aqueous solutions [17]. Shellac is approved in food industry as a food additive. It is characterized by good film-forming properties and is used for coating of citrus fruits, confectionaries and nutritional supplements providing a great barrier to moisture and gases [17–19]. In pharmaceutical sector this natural polymer has been used for sealing, glossing and enteric coating of drug products [20]. However, the application of shellac in pharmaceutical industry has significantly declined [20]. The limitations associated with the use of shellac in pharmaceutical products and in particular in the enteric-coated dosage forms are based on its low solubility in higher pH media (such as intestinal fluid), high batch-to-batch variations and insufficient stability [5,21–24]. The properties of shellac strongly depend on the raw material (seedlac), the refining process, and the parameters during its processing [25]. Therefore, synthetic and semi-synthetic polymers have successfully replaced shellac in enteric coatings in pharmaceutics [9,24]. The use of shellac in drug products is practically limited to moisture barriers and to pharmaceutical printing inks for capsules and tablets [13]. A great alternative to shellac, both in pharmaceutical and in food industry, including its interface (nutraceuticals) may be constituted by lipid-based coatings.

Lipids are a large group of organic compounds and include fatty acids, neutral fats (i.e. triacylglycerols), other fatty-acid esters, soaps, long-chain (or fatty) alcohols and waxes, sphingoids and other long-chain bases, glycolipids, phospholipids, and sphingolipids, carotenes, polyprenols, sterols (and related compounds), terpenes, and other isoprenoids [26]. Lipids belong to the nutrients required for the body to function and maintain overall health [27]. They are delivered to the body with ordinary foods (e.g. with butter, oils, meats, nuts and with many processed foods) [27]. Typically, an adult ingests ca. 60-80 g of lipids daily [28]. The most important lipids, from a nutritional point of view, are: triglycerides (also called triacylglycerols), phospholipids and sterols [27,29]. Triacylglycerols constitute more than 95 percent of lipids in the diet [27].

Lipids already offer a wide range of applications in pharmaceutical formulations. The great advantage of lipids used as pharmaceutical ingredients is their excellent toxicity profile [28]. Lipid excipients are derived primarily from the food industry [30]. Generally, they are digestible and Generally Recognized As Safe (GRAS) [31]. Lipid excipients are found in liquid (parenteral [32] and oral emulsions [33]), semisolid (ointments) and solid dosage forms (tablets, capsules, implants, suppositories [28]). Their applications include their use as emulsifying, solubilizing, stabilizing, stiffening, viscosity-increasing and thickening agents, as tablet and capsule lubricants, tablet binders, skin penetrants, emollients, oleaginous vehicles, ointment bases, solvents and others [13]. They can be used to mask bitter taste and/or prevent the degradation (by light, humidity) of several active substances [28,31]. In lipid-based drug delivery systems they enhance the absorption of poorly soluble drugs improving their bioavailability [34–37]. They can also provide proper protection from

gastric environment for certain sensitive actives [28]. Furthermore, lipids are applied in modified-release (MR) dosage forms in order to sustain or delay the drug release [28]. The sustained drug release in oral solid dosage forms can be achieved by processing with lipid excipients by means of the following methods: direct compression, dry and wet granulation, melt granulation/melt pelletization, hot-melt coatings, molding, spray congealing/chilling and prilling, solid extrusion [30,31,38,39]. As a result, lipid matrices providing extended release of active substances can be produced.

On the market only few oral solid drug products are present, which use lipid excipients for the modification of the drug release. One example is Mucosolvan® retard capsules. Here, the sustained-release of the active substance is assured through the use of carnauba wax with the addition of stearyl alcohol [40]. This drug formulation is produced by high-speed stirring of a melted lipid phase in a hot surfactant solution [30]. The next example of sustained-release drug product is Ibuprofen AL 800 retard tablets which comprise sucrose ester and fatty acid (sucrose dipalmitate and stearic acid) in the tablet cores [41,42]. A very interesting commercial product, where lipid excipient provide a modified-release of the active substance is Lodotra® MR tablets. Lodotra® is a tablet with delayed release behavior [43]. The drug release occurs ca. 4 hours after swallowing [43]. Lodotra® is taken at bedtime, but the active pharmaceutical ingredient is released in the early hours of the morning [43,44]. In this way the API is delivered at the most suitable time for the treatment of the disease [45]. This medication is an example of chronopharmaceutical dosage form [46]. Chronophramceutics are drug products which deliver active substance(s) considering patient's physiological and biochemical conditions [46,47]. The used chronopharmaceutical technology in Lodotra® product is the GeoClock technology [45,46]. GeoClock tablets are prepared via press-coating method [44,48]. The outer tablet layer of Lodotra® consists mainly of hydrophobic Glycerolbehenate with a small amount of PVP as a wicking agent. This shell ensures a pH-independent lag time prior to the drug delivery out of the core [44]. However, there is no product present on the market using lipids in order to provide pH-sensitive drug delivery. Because of lipids properties and their fate in the gastrointestinal tract, they could be potentially used to form pH-sensitive enteric coatings.

Lipids proposed in the current study for the development of pH-sensitive enteric coatings include monoacylglycerols (monoglycerides), diacylglycerols (diglycerides), and fatty acids. Mono- and diglycerides contain in their molecules ester bonds that can be potentially cleaved by lipolytic enzymes occurring in the gastrointestinal tract [49]. Acylglyceroles after intake are digested in the gastrointestinal (GI) tract to fatty acids and 2-monoglycerides. Subsequently these products are solubilized to form a colloidal dispersion within bile salt–lecithin mixed micelles [50,51] . During gastrointestinal lipid digestion three main steps can be distinguished: 1) the dispersion of fat globules into finely divided emulsion particles, 2) the enzymatic hydrolysis of fatty acid esters at the emulsion-

water interface and 3) the desorption and dispersion of insoluble lipid products into an absorbable form. The beginning of the enzymatic lipid hydrolysis already occurs in the stomach [52] and is mainly caused by gastric lipase [53]. However, only from 10 % to maximal 25-30 % of ingested TGs are hydrolyzed here due to the negative feedback mechanism of the fatty acids on gastric lipase [54–56]. The main hydrolysis and absorption occurs instead in the upper small intestine. The upper small intestine provides the required conditions for a complete lipid digestion (less acidic microenvironment, pancreatic lipase, esterases, bile salts, lecithin) and specialized absorptive cells [52]. It could be therefore assumed, that lipid-based coatings with appropriate composition and amount of lipids will constitute a good barrier in gastric environment. Consequently, the release of API in the stomach will not occur or will be insignificant.

Besides digestion the influence of pH values existing in GI tract on lipids also needs to be considered. The pH of the stomach varies from 1 to 2 (fasted state) up to 5-6 (fed state) [8,57]. The pH value of duodenum may range from 5 up to almost neutral values [5,8]. This range results from progressive dilution of acid chyme from the stomach by bicarbonate ions in the pancreatic secretion [5]. In the lower small intestine sections, the pH gradually increases [8]. In the ileocecal region the pH value reaches more than 7 [8]. Due to acidic excretion of colonic bacteria, the pH value in the colon ranges between 6 and 7 [8]. Considering the differences in pH values within the GI tract lipid-based enteric coatings could be formed. Carboxylic groups present in some lipids (fatty acids, triglycerides, other fatty-acid esters) remain non-dissociated (un-ionized) at low stomach pH [52]. The ionization of carboxylic groups depends on the pH and the local microenvironment. For example, the pKa of oleic acid has been reported to be between pH 4 and pH 10 [58]. Within a physiological relevant environment, at least partial deprotonation at neutral pH values in the intestine is expected. It could be therefore anticipated, that the permeability of the lipid-based coatings will grow with increasing pH values. As a result, the release of an active ingredient will take place in intestinal milieu.

In addition to enzymatic activity and pH values existing in the GI tract, gastric emptying should be taken into account when developing enteric drug delivery systems [5]. Gastric emptying is highly variable and strongly depends on the presence or absence of food. In the fasted state the stomach is subject to the migrating myoelectric complex (MMC) forces. In the presence of food, the MMC forces are disrupted and replaced by fed state contractions. Hence, gastric emptying can be hindered [59]. It has been reported that the gastric emptying of coated tablets may take from 30 min to 7 hours [5]. An enteric coated tablet leaving the stomach within dozen minutes may be an ineffective drug delivery system, unless the coating quickly dissolves/disintegrate when contacting the intestine environment [5]. On the other hand, an enteric coating of a tablet residing in the stomach for many hours may not be able to remain intact resulting in the drug release [5].

The variability of gastric emptying can be somewhat overcome by abiding by the recommendations of drug intake (e.g. in fasted state) and by choosing an appropriate dosage form. Multiple-unit dosage forms (MUDF) are characterized by low variability of intra- and inter-subject of drugs absorption, thus showing a more uniform plasma level than monolithic dosage forms [59,60]. This can be explained by their more consistent gastric emptying [61,62]. They leave the stomach more easily under fasted and fed conditions compared to monolithic dosage forms [61]. Small particles, such as pellets, can pass the pylorus also when it is closed [60]. MUDF exhibit even advantages compared to single-unit dosage forms. For instance, here the active substance is divided into numerous of particles so local irritations could be avoided [60,63,64]. In the case of coated monolithic dosage forms, there is a possibility of "dose dumping" [60]. This phenomenon can be almost excluded by coating of multiple-unit dosage forms [60]. It is highly improbable that most of pellet coatings will be simultaneously damaged [60]. Hence, the risk of an uncontrolled drug release can be minimized [60,63]. Multiple-unit dosage forms seem therefore to be the most suitable dosage forms for enteric drug delivery systems.

The crucial step in the development of enteric coatings was the choice of the constituents. The selection of potential coating components was based on the following assumptions:

- Coating formulation should only be composed of edible and biodegradable components (food grade materials);
- Lipids should constitute main ingredients in the coating formulation;
- Due to presence of lipids in the coating material the control of the drug release should be ensured;
- The developed coating should be pH-sensitive;
- No release of the active substance in acidic medium (gastric milieu), for at least 2 hours, should occur;
- Drug release should take place at higher pH values (intestine milieu).

Substances which were used for the formation of lipid-based enteric coatings included: lipids, film-forming polymers, plasticizers, emulsifiers and stabilizers. These substances were matched variously in order to find the most satisfactory composition of the lipid-based coating.

As the main components of enteric coatings, the following lipids were proposed: palmitic acid, stearic acid, mono- and diglycerides esterified with citric acid and mono- and diglycerides esterified with citric and lactic acid.

Straight-chain monobasic carboxylic acids from fats and oils derived from edible sources, to which palmitic acid and stearic acid belong, are accepted as safe for use in food and in the

manufacture of food-grade additives [65,66]. These fatty acids rank among GRAS substances [13,67]. They are used in foods as a plasticizing, lubricating, binding, defoaming agent and flavoring ingredient and as reagent in the manufacture of other food-grade additives [65,68]. In pharmaceutical preparations palmitic acid is used as emulsifying agent, skin penetrant, tablet and capsule lubricant [13]. Stearic acid is used in pharmaceutical preparations as emulsifier, solubilizer and as lubricant for tablets and capsules. This fatty acid may also be used in enteric coatings and as sustained-release drug carrier [13].

Mono- and diglycerides esterified with citric acid (also known as: CITREM, citric acid esters of mono- and diglycerides, citroglycerides or citric and fatty acid esters of glycerol) are widely used in food industry, e.g. as emulsifiers, stabilizers, antispattering agents, flour improvers, sequestrants and as antioxidants [69,70]. These food additives are generally recognized as safe [71]. Their acceptable daily intake is not specified [72]. Citroglycerides are completely hydrolyzed in the intestinal tract. Products of their hydrolysis occur naturally in everyday diet [72].

Mono- and diglycerides esterified with citric and lactic acid belong to liquid emulsifiers whose use is approved in foods [73,74]. They are also suitable for many cosmetic applications. This substance is only based on vegetable feedstock. It contains unsaturated edible fatty acids including linoleic acid and oleic acid [73].

The addition of film-forming polymers was necessary because of poor film-forming properties of lipids. Hydroxypropylcellulose, hydroxypropylmethylcellulose, pullulan and modified hydroxypropyl starch belonged to the film-forming polymers used for the creation of lipid-based coatings.

Hydroxypropylcellulose (HPC) is generally regarded as a nontoxic and nonirritating material [13]. It is approved for use as food additive [75,76]. In foods it is used as an emulsifier, stabilizer and edible coating and film to maintain food quality. It provides a barrier between food and surrounding environment (moisture, gas and solute barriers) [77–79]. This cellulose derivative is also widely applied in pharmaceutical preparations as a coating, emulsifying, stabilizing, suspending, thickening, viscosity-increasing agent and as tablet binder [13]. HPC is also suitable for cosmetic applications [13,76]. A great advantage of HPC is its physiological impassivity. It is neither absorbed from gastrointestinal tract nor metabolized by the human body. In GI tract it acts as a soluble dietary fiber and hence has a positive impact on the digestive system [76]. Although, an excessive oral consumption of HPC may have a laxative effect [13], in usual food applications this laxative concentration is not achieved [76].

Hydroxypropylmethylcellulose (hypromellose, HPMC) is like HPC generally regarded as a nontoxic and nonirritating material and it is approved as food additive [13,80]. HPMC finds a broad application in food industry as a binding, boilout control and film-forming agent [81]. It is also used to

form edible coatings to extend the shelf-life of foods [77–79]. In pharmaceutical formulations it is used, among others, as a coating, dispersing, emulsifying, foaming, stabilizing, controlled-release agent and many others [13]. It has been reported that the consumption of long-chain HPMC types provides some benefits. Harmful or nutritionally unwanted substances occurring in food can be absorbed into the polymer and eliminated from the body. Additionally, HPMC causes delayed sugar resorption enabling the control of the glycemic index [76]. Excessive consumption of hydroxypropyl cellulose, however, may have a laxative effect [13].

Pullulan is a naturally occurring polysaccharide which is applied in foods as a multiple-use direct additive. It is generally recognized as safe. In foods it is used as e.g. emulsifying, stabilizing, thickening, binding, glazing, viscosity-increasing agent. Because of its excellent film-forming properties it is also used to form edible films of fruits in order to provide an oxygen barrier and hence to maintain the quality [82–84]. Pullulan is used in the production of capsules (as a substitute of gelatin) and as a coating of tablets containing dietary supplements [84].

Lycoat® pregelatinized, hydroxypropyl starch is a novel polymer specifically developed for the aqueous film coating for immediate release. The origin of this substance is natural, vegetal and renewable. Lycoat® complies with the European directive on food additives. It is suitable for nutraceutical film coating [85,86].

In order to give an appropriate flexibility to the lipid-based films, the use of plasticizers was considered. The use of emulsifiers and stabilizers was intended to ensure an adequate stability of the coating dispersions. In the first place, emulsifiers, stabilizers and plasticizers which are permitted as food additives were used for the formation of lipid-based enteric coatings. To these belonged: glycerol, starch sodium octenyl succinate, 1-dodecanol. The non-food grade substances (poloxamers, dibutyl sebacate) were used only in a case of unsatisfactory coating formulation properties.

To achieve the goal of the development of a lipid-based enteric coating, a multistep development strategy was developed. In particular, the following experiments were conducted: pH-dependency tests of potential formulation lipids, screenings of casted and sprayed films, fluidized bed coating of pellets, evaluation of coated pellets, differential scanning calorimetry (DSC) analysis.

The pH-dependency tests of potential formulation lipids was carried out to predict the behavior of lipids in gastric and in intestine medium. The results of these tests contributed to the selection of lipid components of the coating formulations.

Screenings of casted and sprayed films included: disintegration tests of casted films, sprayability tests of produced coating dispersions, disintegration tests of sprayed films, and water resistance tests of sprayed films. The disintegration tests of casted and sprayed films aimed to investigate the influence of the pH value on the formed films. Sprayability tests estimated the ability

of the coating dispersions to create films during spraying. Water resistance tests of sprayed films were performed to check the coating permeability in the acidic medium and in simulated intestinal fluids. Only coating dispersions forming films with adequate properties were used for the coating of pellets. The choice of pellets as a dosage form for lipid-based enteric coatings was associated with many advantages of multi-unit dosage forms compared to single-unit dosage forms.

During fluidized bed coating the capability of coating dispersions to film formation around pellets was examined. The performance of multiple coating processes had to optimize coating parameters in order to enable the most effective coating of particles and to prevent unwanted phenomena like pellets agglomeration, lack of film formation.

Coated pellets were analyzed by following tests: evaluation of film properties by means of an optical microscope, pH-dependency tests of coated pellets, film permeability tests, electron paramagnetic resonance (EPR) studies, dissolution tests and differential scanning calorimetry (DSC) analysis. The observation of coated pellets under an optical microscope was performed to evaluate the film surface (e.g. presence or lack of cracks) and its mechanical properties (flexibility, hardness/softness). The pH-dependency tests had to show the impact of the pH value on the film properties during coated pellets incubation in acidic medium and in phosphate buffer. The aim of the film permeability tests was to present the coating permeability in gastric and intestinal milieu. Another technique used for the evaluation of film permeability was EPR spectroscopy. This study allowed to demonstrate the penetration of water and the model drug through the lipid-based coating. Dissolution tests aimed to present the dissolution kinetics of the model drug released from coated pellets in acidic medium and in phosphate buffer. These tests were performed directly after coating of pellets and after their storage for a defined time period under different humidity and temperature conditions. In this way the stability of the lipid-based coatings was estimated. The dissolution tests were also used to investigate the influence of the film layer thickness on the dissolution kinetic of the active substance. The aim of the DSC analysis was to detect interactions between excipients and to study storage stability of lipid-based coatings.

2. Materials and methods

2.1. Materials

A list of substances used in the current work with their function and origin is presented in Table 1.

Table 1. List of substances used during coating formulation development.

Product name	Function	Origin
Avicel PH 101	Pelletization aid [87]	FMC BioPolymer, Belgium
Caffeine	API [88]	Caelo, Germany
Cellets® 1000	Starter cores [89]	Harke Pharma, Germany
Cleargum®CO	Emulsifier/stabilizer [90]	Roquette, France
Dibutyl sebacate	Plasticizer [91]	Sigma Aldrich, Germany
1-Dodecanol	Plasticizer [92]	Sigma Aldrich, Germany
Glycerol	Plasticizer [93]	Carl Roth, Germany
Granulac 200	Extrusion-spheronization [94]	Meggle, Germany
Grinsted® Citrem	Emulsifier/stabilizer [95]	Danisco, Denmark
4-Hydroxy-TEMPO	Spin probe [96]	Sigma Aldrich, Germany
Imwitor® 370 P	Emulsifier/stabilizer [74]	Cremer Oleo Division, Germany
Imwitor® 372 P	Emulsifier/stabilizer [74]	Cremer Oleo Division, Germany
Imwitor® 373 P	Emulsifier/stabilizer [74]	Cremer Oleo Division, Germany
Imwitor® 375	Emulsifier/stabilizer [74]	Cremer Oleo Division, Germany
Kolliphor® P 188	Emulsifier/solubilizer [97]	BASF, Germany
Kolliphor® P 407	Co-emulsifier/consistency enhancer [97]	BASF, Germany
Kollisolv P 124	Solvent [98]	BASF, Germany
Lycoat®	Film forming agent [86]	Roquette, France
Methylene blue	Dye	Sigma Aldrich, Germany
Nisso HPC	Film forming agent [99]	Nippon Soda, Japan
Palmitic acid	Emulsifying agent [13]	Carl Roth, Germany
Pharmacoat	Film forming agent [100]	Shin-Etsu Chemical, Japan
Pullulan	Film forming agent [101]	Hayashibara, Japan
Stearic acid	Emulsifying agent [13]	Sigma Aldrich, Germany
Sudan Red	Dye	Sigma Aldrich, Germany

In the course of development of coating formulations, various plasticizers, stabilizers, emulsifiers and film forming polymers were examined. In the course of fluidized bed coating commercially manufactured placebo MCC spheres as well as self-prepared pellets were used. For pellets preparation a mixture of extrusion-spheronization aids with an active pharmaceutical ingredient were utilized. Methylene blue, as a hydrophilic dye, was applied to illustrate film permeability. Lipophilic dye-sudan red- facilitated the observation of film formation.

2.2. Methods

2.2.1. PH-dependency tests of potential formulation lipids

The pH-sensitivity of potential lipids was investigated as follows: 0.1 g of lipids were placed in test tubes filled with 6 ml of dissolution media. Two different dissolution media were used: hydrochloric acid medium (pH 1.2) and phosphate buffer (pH 6.8). The test tubes were put into a chamber of end-over-end mixer, where the temperature was maintained at 37 ± 0.5 °C. The tubes were rotated during the experiment. The behavior of the lipids in dissolution media was observed every 15 minutes. Afterwards the pH-sensitivity of lipids was evaluated.

The end-over-end mixer is shown in Figure 1. This device was also used for the analysis of pH-dependency of casted films and disintegration tests of sprayed films.

End-over-end mixer.

Thermostatic chamber.

Figure 1. Presentation of end-over-end mixer.

2.2.2. Preparation of coating dispersions

The overall presentation of preparation of coating dispersions is shown in Figure 2. Hydrophilic phase was heated to 80°C. Meanwhile lipophilic phase was melted over a water bath. Both phases were mixed and homogenized for 5 minutes using an Ultra-Turrax homogenizer (IKA Ultra-Turrax® T 18 basic). The obtained dispersion was then cooled down to ambient temperature. Finally, the film-forming dispersion was degassed with a vacuum pump. The composition of hydrophilic and lipophilic phase was specific for the coating formulation. The following belonged to hydrophilic substances: water, film forming polymers, hydrophilic plasticizers and emulsifiers. Lipophilic substances included: lipids, lipophilic plasticizers and emulsifying agents, sudan red dye.

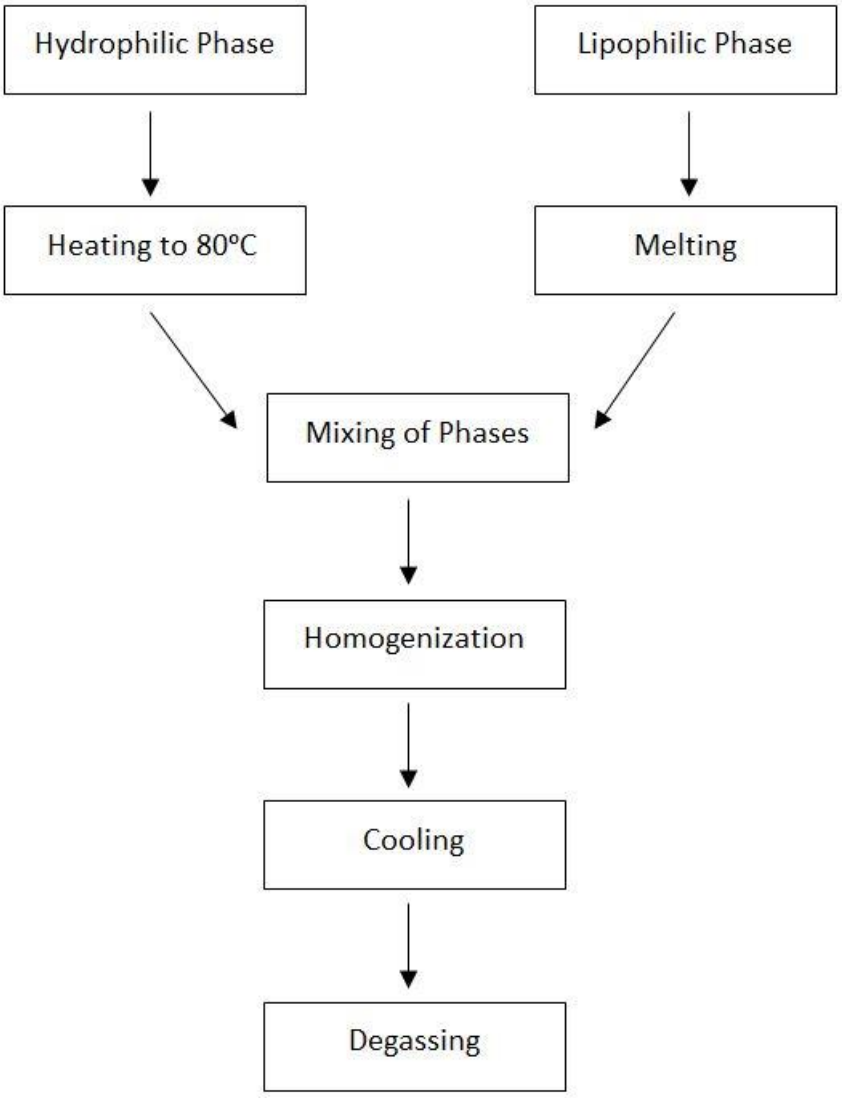


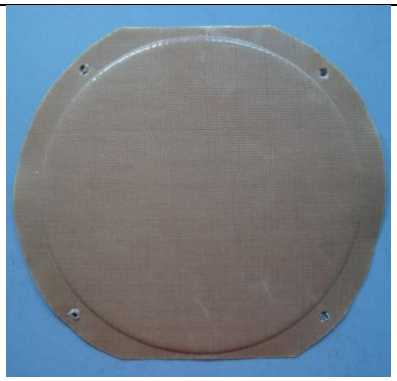
Figure 2. Overall scheme of manufacture of coating dispersions.

2.2.3. Screening of films

2.2.3.1. Preparation of casted films

10 ml of produced coating dispersion was casted on a polytetrafluoroethylene surface (PTFE), placed on a metallic plate (diameter=10 cm, see Figure 3). The film was then dried at room temperature for ca. 16 hours. If necessary, the drying process took up to 24 hours.

Metallic plate covered with polytetrafluoroethylene film.



Polytetrafluoroethylene film placed on a metallic plate.

Figure 3. Presentation of metallic plate used for casting of coating dispersions.

2.2.3.2. Disintegration tests of casted films

Pieces ca. 1 cm per 1 cm of casted films (Figure 4) were placed in test tubes filled with 6 ml of dissolution media. Two dissolution media were used: hydrochloric acid medium (pH 1.2) and phosphate buffer (pH 6.8). Subsequently, the test tubes were placed in an end-over-and mixer. During the test the temperature was hold at 37 ± 0.5°C. The behavior of the film pieces was controlled every 5 minutes.

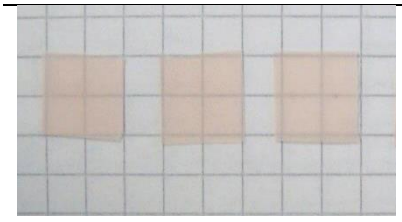


Figure 4. Pieces of casted film.

2.2.3.3. Sprayability tests

The spraying process was performed as follows: a coating dispersion was placed on a magnetic stirrer and mixed during the whole spraying process. The stirring ensures homogeneous distribution of the solids. The coating dispersion was delivered through an inner tube to a nozzle by means of a pump. Through the outer tube air under pressure was delivered. The liquid was sprayed on a polytetrafluoroethylene (PTFE) surface fixed to a rotating roller. The heated air was moved from the bottom up. One temperature sensor was placed nearby the air inlet. The other temperature sensor and humidity sensor were located next to the air outlet. Once the required amount of coating dispersion was sprayed, the drying process began. The schematic illustration of the spraying device is presented in Figure 5. This device was made at our university (developed by Martha Heider).

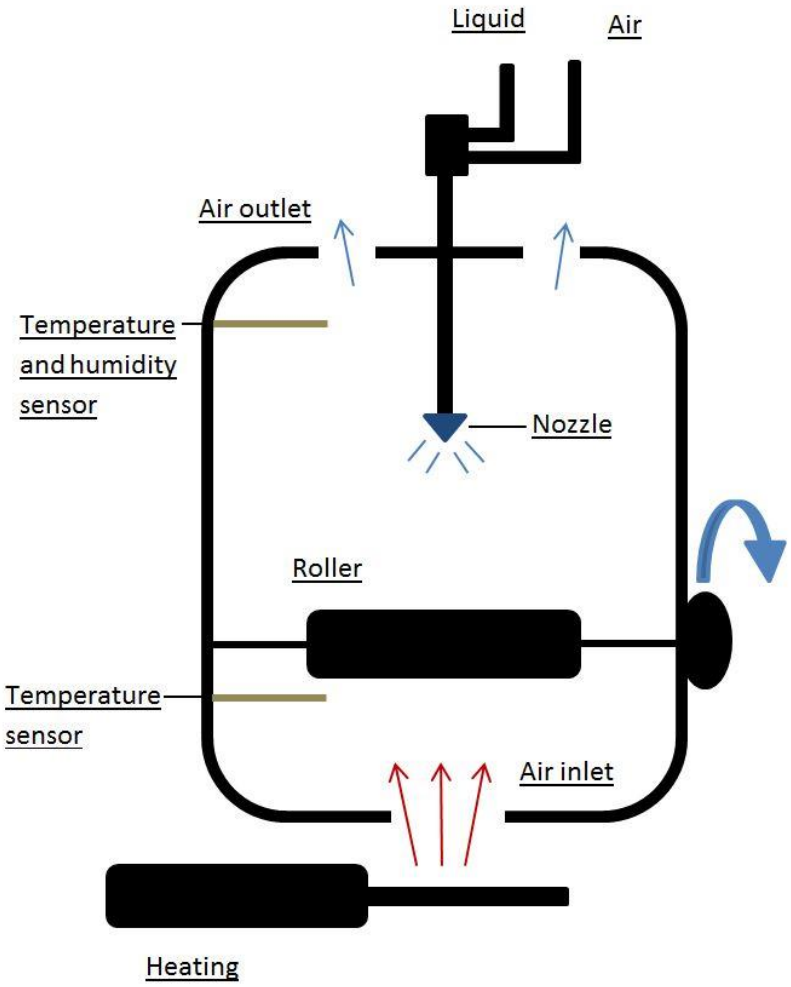


Figure 5. Presentation of a spraying device used for sprayability tests.

During the spraying process the adjustment and control of some process parameters was possible. These parameters are listed in Table 2. The parameter settings were dependent on dispersion features. For instance, dispersion with higher viscosity required higher Atomizing Air Pressure to obtain fine droplets; or dispersions with higher amount of water required higher inlet air temperature to ensure proper coalescence during film formation etc.

Table 2. Parameters adjusted during spraying process.

Parameter	Unit
Atomizing Air Pressure	bar
Rotation of a roller	rpm
Spray Rate	g/min
Inlet Air Temperature	°C
Outlet Air Temperature	°C
Relative Humidity	%

2.2.3.4. Disintegration tests of sprayed films

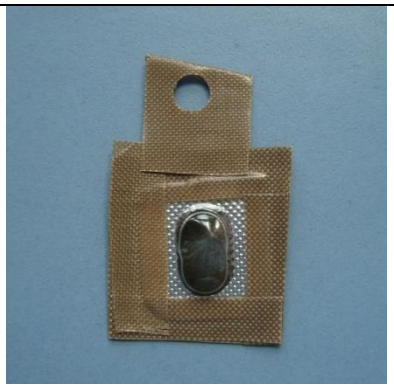
The disintegration tests of sprayed films were performed in an end-over-end mixer (see Figure 1). The experiments' temperature was maintained at 37 ± 0.5°C. First, pieces of sprayed films were placed in 6 ml hydrochloric acid medium (pH 1.2). If no disintegration of the film after 2 hours' incubation in HCl medium occurred, the piece of the film was shifted to phosphate buffer (pH 6.8). The observations of the test were carried out every 5 minutes. The disintegration times were noted.

2.2.3.5. Water resistance tests of sprayed films

For the water resistance tests a special film holder with a dye container was developed (Figure 6). As a dye methylene blue was used. If a tested film was permeable to water, the dissolution of the dye occurred. Subsequently, the dissolved dye penetrated through the film into the dissolution medium. As a consequence, the color of the dissolution medium changed.

During each water resistance test a piece of sprayed film was at first incubated for 2 hours in acidic medium (pH 1.2). If the color of the HCl medium did not change, the film was then shifted to dissolution medium with pH value of 6.8 (phosphate buffer, FeSSIF without pancreatin, FeSSIF with pancreatin).

Piece of a film (at the front).



Container with a dye methylene blue (at the back).

Figure 6. Presentation of a dye container.

Water resistance test in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8)

A piece of sprayed film was fixed to the developed film holder with the dye container. Then, it was placed in a beaker filled with a dissolution medium. The beaker was in a water-bath (Figure 7). The medium temperature was maintained at 37 ± 0.5°C. The medium was continuously stirred during the test. For the first 2 hours' the film was incubated in hydrochloric acid medium (pH 1.2). After 2 hours incubation the medium was changed to phosphate buffer (pH 6.8). The sample was controlled every 5 minutes. The change of the color of the dissolution medium to blue indicated film permeability.

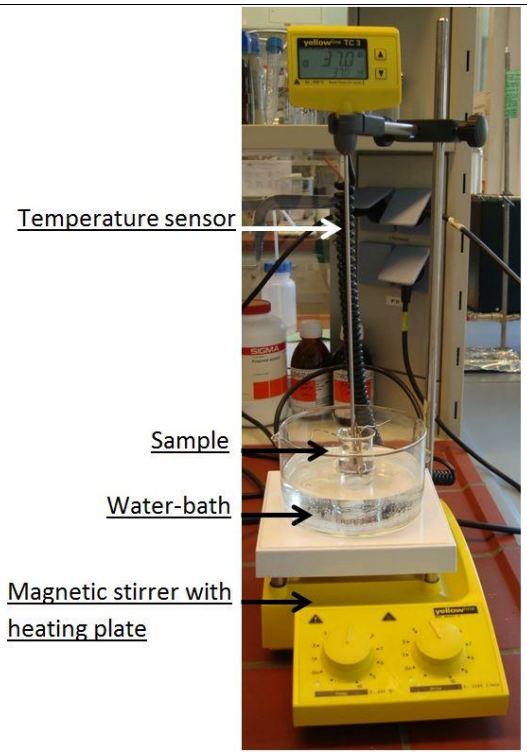


Figure 7. Experimental setup of the water resistance test.

Water resistance test in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (without pancreatin, pH 6.8)

This test was performed in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (FeSSIF, pH 6.8) without the addition of pancreatin. The composition of FeSIFF with and without pancreatin is listed in Table 3.

Table 3. Composition of Fed State Simulated Intestinal Fluid (FeSSIF) with and without the addition of pancreatin.

Composition	FeSSIF (without pancreatin)	FeSSIF
Bile salts [mM]	15	15
Phospholipids [mM]	3,75	3,75
Sodium chloride [mM]	150	150
Calcium chloride [mM]	5	5
Tris-solution [mM]	5	5
Pancreatic lipase activity [U/ml]	-	450

The measurement conditions of this test were the same as for the water resistance test performed in hydrochloric acid medium and in phosphate buffer (pH 6.8, see Figure 7). The film holder was fixed to a beaker placed on a magnetic stirrer in water bath. The water bath maintained constant temperature during the test (37 ± 0.5°C). The dissolution media were continuously stirred during the test. For the first 2 hours the film was incubated in hydrochloric acid medium (pH 1.2). If no change of the HCl medium color occurred, the film was next placed in the Fed State Simulated Intestinal Fluid (FeSSIF without pancreatin, pH 6.8). The color of FeSSIF was observed every 5 minutes. The initial color of the FeSSIF was yellow. The change of the color to green indicated film permeability.

Water resistance test in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (with pancreatin, pH 6.8)

The sprayed film fixed to the developed film holder (Figure 6) was incubated for 2 hours in hydrochloric acid medium (pH 1.2). Subsequently, the incubation was performed in Fed State Simulated Intestinal Fluid (FeSSIF with pancreatin, pH 6.8). The test temperature was maintained at 37 ± 0.5°C. The dissolution media were continuously stirred during the test. The color of dissolution media was observed every 5 minutes. The initial color of the FeSSIF medium was yellow. In the case of film permeability the medium color became green. The composition of FeSSIF is listed in Table 3.

2.2.4. Differential Scanning Calorimetry analysis

The differential scanning calorimetry measurements were performed on a DSC 200 (Netzsch GmbH, Selb, Germany). The sample was placed in a closed pan and was heated from 0°C to 90°C. The heating rate was 10°C/min. Nitrogen was used as a flushing gas with a flow rate of 10 ml/min. After heating the sample was cooled down. Subsequently, the second heating was performed.

2.2.5. Fluidized bed coating

The coating process was performed in the Mini-Coater MFL.01 with a Wurster insert (Vector Corp., Marion, USA). The coating device is presented in Figure 8.

The mass of 10 g or 15 g of pellets were placed in the coating container. Pellets were heated until the required outlet air temperature was achieved. The coating dispersion was placed on a magnetic stirrer and was continuously stirred to avoid sedimentation. The coating dispersion was delivered by means of a pump to the nozzle through a heated tube. The coating process lasted until the proper amount of the coating dispersion was sprayed. The final stage was the drying of pellets. The drying process was performed gradually. The inlet air temperature was reduced by 10°C every 20 minutes until room temperature was achieved. The whole coating process was constantly monitored. Thereafter, the coated pellets were weighed and subjected to further analysis.

During the coating process the following parameters were monitored and adjusted (Table 4):

Table 4. Parameters of coating process.

Parameter	Unit
Atomizing Air Pressure (Nozzle Air)	psi
Air Flow (Air Volume)	LPM; L/min
Inlet Air Temperature	°C
Outlet Air Temperature	°C
Spray Rate (Pump Output)	g/min

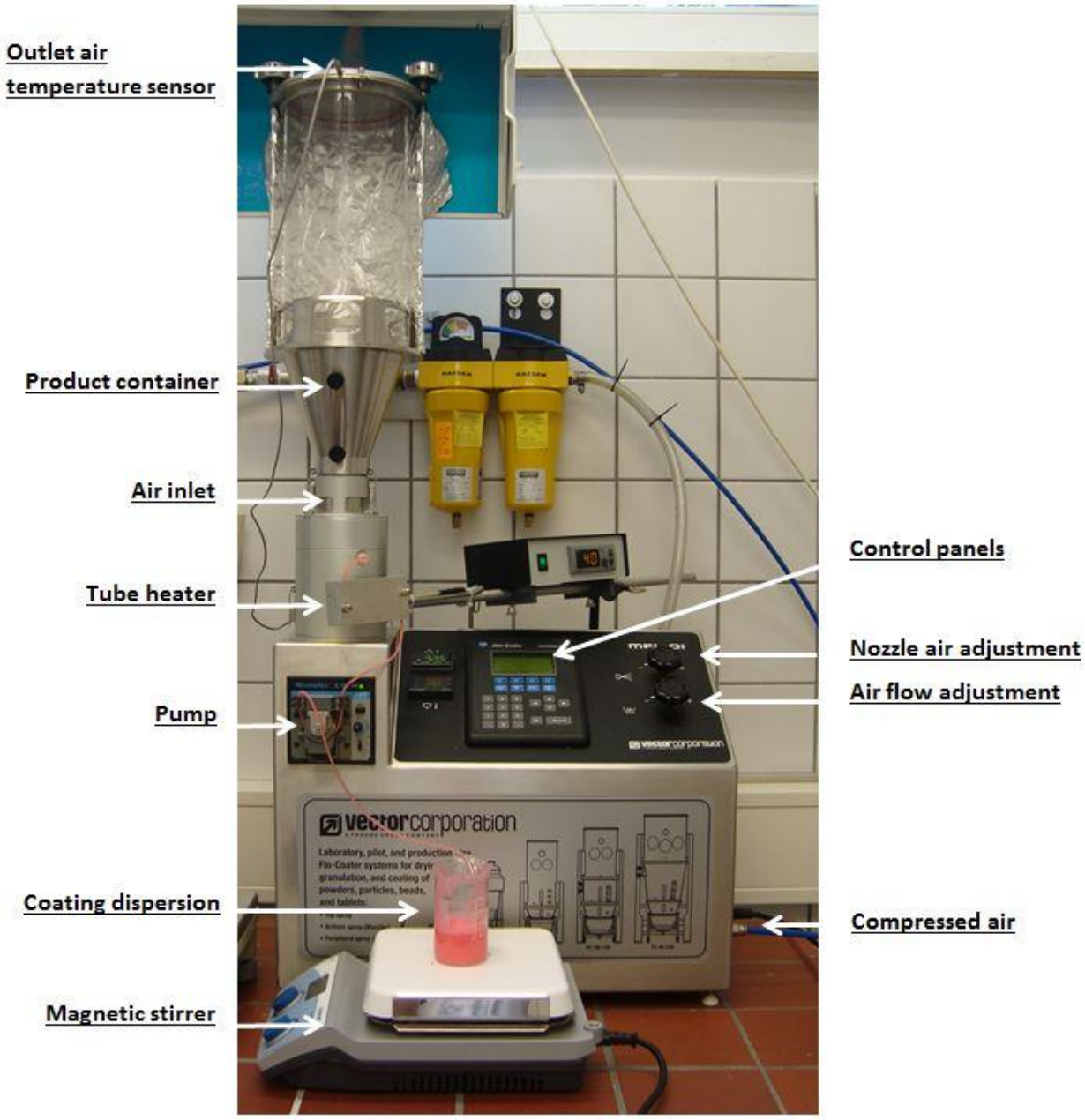


Figure 8. Photograph of the Mini-Coater MFL.01 (Vector Corp., Marion, USA) used for the coating process.

2.2.6. Preparation of methylene blue-microcrystalline cellulose pellets

The mass of 0.2 g of methylene blue was dissolved in 50 g distilled water. Then 30 g of microcrystalline cellulose pellets (Cellets® 1000) were placed in the methylene blue solution and stirred occasionally (room temperature). After 2 hours the pellets were withdrawn and dried in a compartment dryer for 2 hours at 60°C. Subsequently, the pellets were cooled down to room temperature. The methylene blue-MCC pellets are presented in Figure 9.

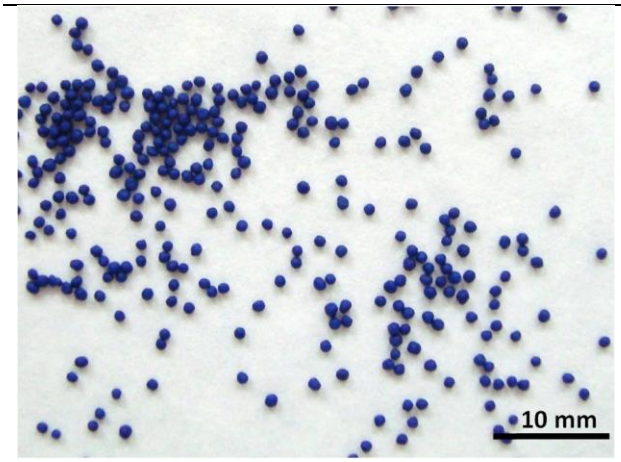


Figure 9. Methylene blue-microcrystalline cellulose (MCC) pellets.

2.2.7. Preparation of 4-hydroxy-TEMPO-microcrystalline cellulose pellets

The mass of 0.01 g of 4-hydroxy-TEMPO (tempol) was dissolved in 20 g distilled water. 15 g of microcrystalline cellulose pellets (Cellets® 1000) were placed in the prepared tempol solution and stirred occasionally for ca. 1.5 hours (room temperature). Afterwards, the pellets were dried in a compartment dryer for about 11 hours at 70°C.

2.2.8. Preparation of caffeine-microcrystalline cellulose pellets (saturation technique)

The mass of 20 g of microcrystalline cellulose pellets (Cellets® 1000) were placed in ca. 73 g of a saturated caffeine solution (caffeine solubility in water at 20°C 20 g/l [88]). The pellets were kept in the caffeine solution for about 12 hours. Subsequently, they were dried in a compartment dryer at 70°C for ca. 12 hours.

The pellets obtained through the saturation technique were used only for screening purposes.

2.2.9. Preparation of caffeine pellets via extrusion/spheronization method

Microcrystalline cellulose, lactose monohydrate and caffeine were mixed for 10 minutes in a planetary mixer (Kenwood). Thereafter, the powder mixture was granulated for 10 min by means of the same planetary mixer. Water was used as granulating liquid. Next the wet mass was extruded at an extrusion speed of 65 rpm using a radial twin screw extruder (Fuji Denki Kōgyō); thickness: 1 mm, perforation diameter: 1 mm. The extrudates were spheronized for 5 min at 600 rpm in a spheronizer (Fuji Denki Kōgyō) equipped with a 3x3 mm^2 cross-hatched friction plate 250 mm in diameter. Afterwards, pellets were dried in a compartment dryer at 40°C. The composition of produced pellets is listed in Table 5.

Table 5. Composition of pellets prepared per extrusion/spheronization method.

Pellets composition	Content [%]
Microcrystalline cellulose	47,75
Lactose Monohydrate	47,75
Caffeine	13,50

2.2.10. Pellets characterization

2.2.10.1. Sieve analysis

Sieving was performed in order to determine particle size distribution of pellets prepared per extrusion/spheronization technique and to separate pellet fractions. Only pellets from a class range of 1000-1400 µm were used for further investigations.

Pellets were sieved using 2000, 1400, 1000, 710, 500, 355 and 250 µm sieves (Retsch, Haan, Germany) on a sieve shaker (Retsch, Haan, Germany) until a constant mass on each sieve was achieved. Afterwards, the percentage content of each fraction was calculated.

2.2.10.2. Evaluation of film properties by means of an optical microscope

For the coating evaluation on pellets surface Olympus SZX 9 microscope was used. The microscope was connected with a digital camera attached to a computer using Olympus Stream 1.6 image analyzing software. Under the microscope the film surface, the mechanical strength and flexibility of the film were evaluated. In order to evaluate the mechanical strength the coated

pellets were subjected to mechanical stress and observed under the microscope once again. To investigate the flexibility, the film was removed from the pellets surface and straightened. Subsequently, the film surface was observed under the microscope.

2.2.10.3. PH-dependency tests of coated pellets

Placebo microcrystalline cellulose pellets were coated with the lipid formulation. Then pellets were placed in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8) for 2 hours. The incubation temperature was maintained at 37 ± 0.5°C. After incubation pellets from these two dissolution media were withdrawn and their appearance was observed and compared under the microscope.

2.2.10.4. Film permeability tests

Film permeability test of coated methylene blue-MCC pellets in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8)

The mass of 0.1 g of coated methylene blue-microcrystalline cellulose (MCC) pellets were parallel placed in two dissolution media, namely in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8). The temperature of the tested media was maintained at 37 ± 0.5°C. Subsequently, the change of the media color was observed. The test was performed in triplicate for each dissolution medium. For easier observation of the dye release modification from coated pellet, uncoated pellets were simultaneously incubated (blank positive sample).

Film permeability test of coated methylene blue-MCC pellets in Fed State Simulated Intestinal Fluid (pH 6.8) without addition of pancreatin

The mass of 0.1 g of coated methylene blue-microcrystalline cellulose (MCC) pellets were incubated for 2 hours in hydrochloric acid medium (pH 1.2) at 37 ± 0.5°C. Thereafter, the pellets were withdrawn and incubated at 37 ± 0.5°C in Fed State Simulated Intestinal Fluid (FeSSIF) without an addition of pancreatin (pH 6.8). During the incubation the change of the media color was observed. The test was performed for a tested formulation in triplicate. Simultaneously, uncoated methylene blue-MCC pellets were incubated in FeSSIF (blank positive samples) under the same conditions. The blank positive samples were used to reveal differences in the dye release between uncoated and coated pellets. The whole composition of the simulated intestinal fluid is listed in Table 3.

Film permeability test of coated methylene blue-MCC pellets in Fed State Simulated Intestinal Fluid (pH 6.8) with pancreatin

The mass of 0.1 g of coated methylene blue-microcrystalline cellulose (MCC) pellets were incubated for 2 hours in hydrochloric acid medium (pH 1.2, 37 ± 0.5°C). Afterwards, the pellets were withdrawn, placed in Fed State Simulated Intestinal Fluid (FeSSIF) containing pancreatin (pH 6.8) and incubated at 37 ± 0.5°C. During the incubation the change of the media color was observed. The test was performed in triplicate for a tested formulation. At the same time uncoated methylene blue-MCC pellets were tested in FeSSIF under the same conditions (blank positive samples). The blank positive samples were used to compare the differences in the dye release between the coated and uncoated pellets. The full composition of the simulated intestinal fluid is listed in Table 3.

2.2.10.5. Electron paramagnetic resonance studies

For the electron paramagnetic resonance (EPR) studies a L-band EPR spectrometer (Magnettech GmbH, Berlin, Germany) working at a microwave frequency of about 1.3 GHz was used. Prepared 4-hydroxy-TEMPO-microcrystalline cellulose (MCC) pellets were coated with the lipid-based coating formulation. The conditions of the coating process are described in Chapter 2.2.5. The mass of 0.26 g of coated pellets was placed in cylindrical shape micro-filter-candle-blanks (porosity "0", ROBU®, see Figure 10). After pellets placement, the filter candles were closed with an impermeable stopper. Next, the filters were put in plastic test tubes filled with 12 ml of the dissolution medium. The test tubes were then incubated in an end-over-end mixer. The temperature of the test was maintained at 37 ± 0.5 °C. As dissolution media hydrochloric acid medium (pH 1.2) and phosphate buffer (pH 6.8) were used. The EPR measurements were first carried out every 0.5 hour. After 1.5 hours of pellets incubation the EPR measurements were performed every 1 hour. The last measurement was carried out after 4 hours of samples' incubation. Both dissolution media were also subjected to EPR measurements at the same time intervals. Parameters of the EPR experiment are listed in Table 6.

Table 6. EPR measurement parameters.

Parameter	Value
B_0 field	49.0 mT
Scan range	12 mT
Scan time	120 s
Modulation amplitude	0,1250 mT

Dimensions of used micro-filter-candles are presented below (Figure 10). The pore size "0" is equivalent to 160 – 250 µm.

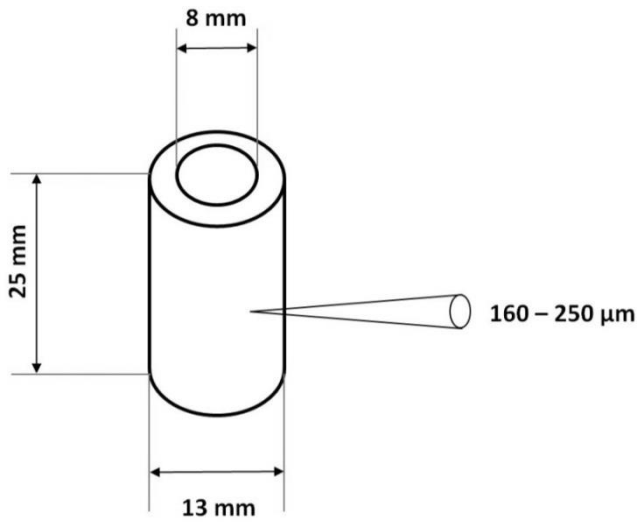


Figure 10. Micro-filter-candle-blanks (ROBU®); porosity "0".

2.2.10.6. Dissolution tests

The dissolution tests were performed according to the Ph. Eur. paddle apparatus (PHARMA TEST, Hainburg, Germany) at a rotational speed of 100 rpm and at temperature of 37°C. This apparatus is equipped with eight vessels and connected with an UV spectrophotometer (J&M Tidas, SA500). As dissolution media hydrochloric acid medium (pH 1.2) and phosphate buffer (pH 6.8) were used. The volume of the dissolution media was each time 900 ml. The quantity of the analyzed pellets was consistent with the sink conditions and amounted to 400 mg. The samples were automatically withdrawn at defined intervals (1-3 h at 5 min, 3 h at 10 min, 4-7 at 30 min, 7-11 h at 120 min) then analyzed spectrophotometrically at 273 nm (λ_{max} of caffeine). After assay of the dissolution samples they were returned to the vessels. Each batch was analyzed in triplicate in each medium.

3. Results and discussion

3.1. Development of lipid-based coating formulation

The development of lipid-based coating formulation included: selection and screening of potential formulation components, development of coating dispersion, analyses of casted film, sprayability tests, analyses of sprayed films and fluidized bed coating of pellets.

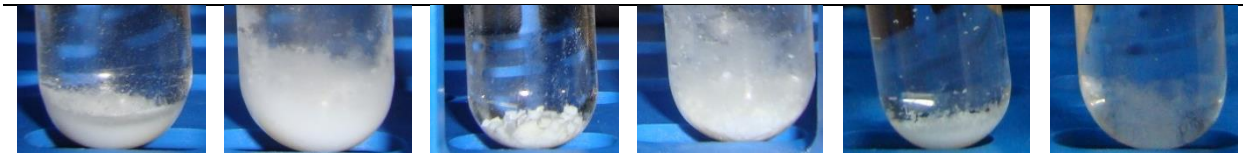
At first, the behavior of various lipids in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8) was investigated. The aim of this experiment was to give the first idea about the possible behavior of the lipid-based coating in gastrointestinal tract. Proper lipids stability in acids will ensure an adequate coating stability in gastric milieu. Thus, no release of the drug will occur (lag phase). On the other hand, the lack of lipids stability at higher pH values will lead to API release in the intestine. Considering the stability of lipid-based coating in GI tract it should also be noted that lipids can interact with mixed micelles (e.g. monoglycerides and fatty acids) and be degraded by digestive enzymes. The lipid digestion begins already in the stomach. However, the main digestion takes place in the small intestine. Therefore, in order to guarantee proper coating stability in the stomach and – simultaneously - the drug release in the intestine, an appropriate coating composition and an adequate amount of lipids are necessary. Another aspect which should also be taken into account by lipids selection is their melting points. Melting points play a major role in the production process of coating dispersion. The higher is the melting point of lipid the more complicated is the production (use and maintenance of high temperature during coating dispersion manufacturing, providing large amount of energy, increase in costs etc.).

As potential components of coating formulation the following lipids were investigated: stearic acid, palmitic acid and various mono- and diglycerides esterified with citric acid (citroglycerides). Melting points of these lipids are presented in Table 7.

Table 7. Melting points of lipids.

Lipid	Melting point °C
stearic acid	66-69 [102]
palmitic acid	61-63 [103]
mono- and diglycerides esterified with citric acid	57-63 [74]

Mono- and diglycerides esterified with citric acid showed pH-dependent behavior. In HCl medium their appearance did not change and they remained solid. In contrast, in phosphate buffer the texture of these lipids changed. They became soft and "cloud– like". Thus it can be assumed that a coating based on citroglycerides will reveal pH-sensitivity. In acidic milieu the coating will demonstrate impermeability, whereas at intestinal pH values the drug release will take place. The behavior of investigated mono- and diglycerides esterified with citric acid in different pH values are shown in Figure 11.



A) pH 1.2 A) pH 6.8 B) pH 1.2 B) pH 6.8 C) pH 1.2 C) pH 6.8

Figure 11. Behavior of mono- and diglycerides esterified with citric acid in HCl medium (pH 1.2) and in phosphate buffer (pH 6.8); A-Imwitor® 370 P, B- Imwitor® 372 P, C- Imwitor® 373 P.

Stearic acid and palmitic acid remained unchanged both in HCl medium and in phosphate buffer. They showed no pH-dependent behavior at this temperature. The calculated pKa value of stearic acid and palmitic acid is 4.95 [104]. This value indicates that at pH 6.8 (phosphate buffer medium) the deprotonated acid fraction will dominate over the protonated form. Therefore a different appearance of these fatty acids in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8) should be expected. However, the ionization behavior of long chain fatty acids is a very complex issue. The pKa values of fatty acids strongly depends on the environment (e.g. buffer composition, ion strength, temperature) and may range from 4.2 to 10.15 [58]. The microenvironment has also a strong impact. For example, in the presence of lecithin, the pKa of oleic acid is in the physiological pH range [58]. The lack of visible changes of the fatty acids at pH 6.8 could be explained. An addition of pH-insensitive lipids in a coating formulation (e.g. palmitic acid and stearic acid) will lead to its greater water resistance. In this manner, through the use of pH-independent lipids in appropriate quantities the duration of the lag phase can be determined.

Besides lipids, other substances were also examined. Lipids alone cannot form a coating with suitable properties. They are usually very poor film formers. Pure lipid films are very brittle and inflexible. Therefore, an addition of further excipients was necessary. The other ingredients included: film-formers, plasticizers and stabilizers. Film-formers are defined as polymers able to harden to a coherent film [105]. The most common film formers used in solutions or dispersions for all pharmaceutical purposes (e.g. taste masking, barrier and controlled-release) contain cellulose ether and ester derivatives, and polymethacrylates [106]. However, in the current study only biodegradable and nontoxic polymers were used for screening purposes. Polymethacrylates were excluded as being purely synthetic and non-biodegradable. Investigated polymers comprised:

pullulan, modified starch, hydroxypropylmethylcellulose (HPMC) and hydroxypropylcellulose (HPC). Nevertheless, film forming polymers often required an addition of plasticizers giving proper flexibility to the coating. Plasticizers used in experimental formulations were: dodecanol, dibutyl sebacate and glycerol. The last pharmaceutical additives investigated were stabilizers. Their addition was necessary to obtain a stable coating dispersion. Adequate dispersion stability leads to good film formation and enables the storage of the dispersion. The following stabilizers were tested: poloxamer 188, poloxamer 124, poloxamer 407 and modified starch ((OSA) starch). All substances used for screening during coating formulation development are listed in Table 8.

Table 8. Substances used for screening during coating formulation development.

Lipids	Film forming polymers	Plasticizers	Emulsifiers/Stabilizers
stearic acid	pullulan	dodecanol	poloxamer 188
palmitic acid	modified starch	dibutyl sebacate	poloxamer 124
mono- and diglycerides esterified with citric acid	HPMC	glycerol	poloxamer 407
	HPC		OSA starch

3.1.1. Development of coating dispersion

The development of coating dispersion was a very complex process. It included the production and evaluation of the coating dispersion as well as the estimation of formed films. On the one hand, the coating dispersion was to demonstrate good properties (homogeneity, stability, viscosity etc.) and simultaneously it should be able to form a coherent film. On the other hand, the formed film should show adequate mechanical strength and flexibility. In addition, the created coating should demonstrate desired pH sensitivity.

The complexity of the coating dispersion development is presented in Figure 12. First of all, the coating dispersion was created from the selected excipients (see Table 8). Next, the properties of the dispersion were evaluated. If inadequate properties were detected, some excipients were replaced by other pharmaceutical additives or their amount in the formulation was changed. If the dispersion was stable, homogeneous and had proper viscosity, it was casted on a polytetrafluoroethylene (PTFE) surface. Afterwards, the properties of the casted film were

estimated (see Chapter 3.1.3). If the film characteristic was unsatisfactory, further modification of the dispersion composition was necessary. In the case of good properties of a casted film, the dispersion was sprayed on a PTFE surface by means of a spraying device (Figure 5). So, the sprayability of dispersion could be estimated (see Chapter 3.1.4). Non sprayable dispersions required additional adjustments of their composition and renewed evaluation. Sprayed film formed from the created dispersion underwent further analyses (see Chapter 3.1.5). The description of the dispersion and of film properties is listed in Table 9.

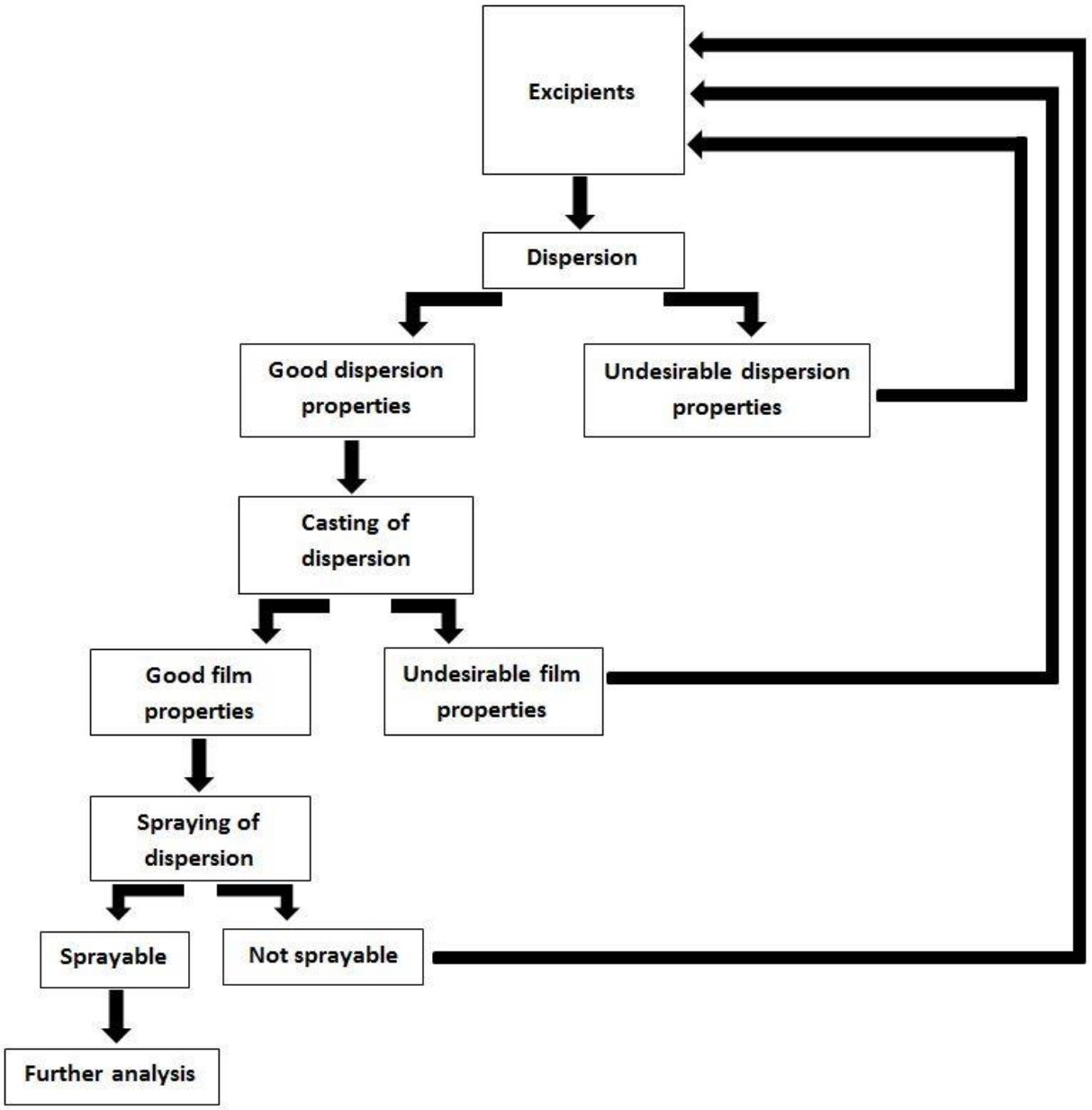


Figure 12. Schema of coating formulation development.

Table 9. Dispersion and film properties.

Good dispersion properties	stable, homogeneous, proper viscosity, sprayable
Inadequate dispersion properties	lack of stability, non-homogeneous, too high viscosity, non sprayable
Good film properties	proper mechanical strength, flexible, homogeneous, adequate water resistance (stability for 2 h in acids (pH 1.2) and disintegration in phosphate buffer (pH 6.8))
Inadequate film properties	non-homogeneous, brittle, low mechanical strength (soft films), low or lack of water resistance (lack of stability for 2 h in acids (pH 1.2))

During the development of coating formulation the production technology of the coating dispersion was also developed and optimized. The following belong to the most important parameters of the manufacturing technology: process temperature, the order of addition components and the time of stirring. As a result a simple, inexpensive and reproducible method was developed.

3.1.2. Screening techniques of manufactured films

In order to evaluate the properties of formed films some screening techniques were developed. The screening methods include: disintegration tests of casted films (in HCl medium and in phosphate buffer, see Chapter 3.1.3), as well as tests performed on sprayed films: disintegration and water resistance tests (in HCl medium, in phosphate buffer, in FeSSIF with and without the addition of pancreatin; see Chapter 3.1.5). The presentation of screening methods and desirable film properties are listed in Table 10. The delay of film disintegration and/or of the change of the medium color indicated the occurrence of the off-release period (lag phase).

First, the visual evaluation of casted films was performed. If a casted film was flexible, homogenous and demonstrated good mechanical strength, it was then subjected to disintegration tests. Unfortunately, many of the films formed were characterized by insufficient stability in acidic medium. Therefore, further modifications of composition of coating dispersions were required. If a film disintegrated too fast during the test, the content of hydrophilic substances was reduced and/or the percentage of lipophilic components was increased. Hereby, the increase of water resistance was observed.

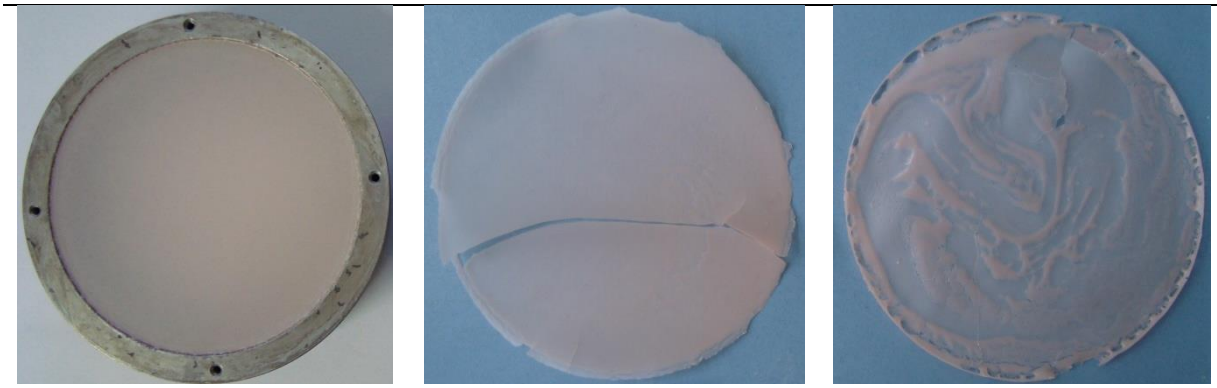
Dispersions forming films showing pH-dependent behavior (no disintegration in HCl medium and disintegration in phosphate buffer) were sprayed on a PTFE surface. Thus, the sprayabillity of the dispersions was evaluated. Subsequently, the sprayed films were taken for further analyses. However, in a case of non-sprayable dispersion, the renewed alterations of its composition were necessary.

Table 10. Presentation of screening methods of casted and sprayed film and desirable film properties.

Screening methods of manufactured films	**Desired properties**
Casted films	
Disintegration test	2 h stability in HCl medium (pH 1.2), disintegration in phosphate buffer (pH 6.8)
Sprayed films	
Disintegration test	2 h stability in HCl medium (pH 1.2), disintegration in phosphate buffer (pH 6.8)
Water resistance test in HCl medium and in phosphate buffer	No color change of HCl medium for 2 h, Color change of phosphate buffer to blue
Water resistance test in HCl medium and in FeSSIF without pancreatin	No color change of HCl medium for 2 h, Color change of FeSSIF to green (slower color change compared to FeSSIF with pancreatin)
Water resistance test in HCl medium and in FeSSIF (with pancreatin)	No color change of HCl medium for 2 h, Color change of FeSSIF to green (faster color change compared to FeSSIF without pancreatin)

3.1.3. Analyses of casted films

After the preparation of a coating dispersion exhibiting good properties (see Table 9), it was casted on a PTFE film placed on a metallic plate (diameter=10 cm). Examples of casted films are presented in Figure 13.



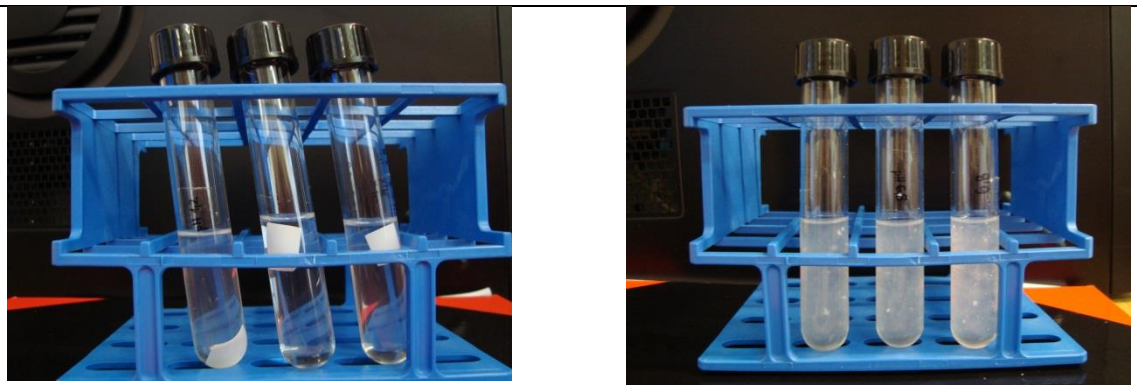
Casted film with proper characteristic.

Brittle casted film.

Non-homogeneous and brittle casted film.

Figure 13. Examples of casted films.

If a casted film was homogenous and demonstrated proper mechanical strength as well as adequate flexibility, its behavior in different dissolution media was analyzed (disintegration test). An example of the disintegration test of one of the developed film formulation is presented in Figure 14. This film showed stability for 2 hours in HCl medium. In contrast, in phosphate buffer a fast disintegration of the film occurred.



pH 1.2

pH 6.8

Figure 14. Example of disintegration test of casted film.

3.1.4. Sprayability tests

The coating dispersion, whose casted film demonstrated adequate mechanical properties, no disintegration within 2 hours in HCl medium and fast disintegration in phosphate buffer (pH 6.8), was sprayed on a PTFE surface by means of a spraying device (Figure 5). Hereby, the sprayability of the coating dispersion was evaluated. If the spraying process was not successful, further modifications of the dispersion composition were necessary. In the case of too high dispersion viscosity, the amount of the soluble film forming polymer was reduced or another film forming polymer with lower viscosity value was used. Subsequently, the sprayed film was subjected to further analysis (see Chapter 3.1.5). Examples of sprayed films are shown in Figure 15.

Homogeneous sprayed film.	Non-homogeneous sprayed film (viscosity too high).	Non-homogeneous sprayed film (viscosity too high).

Figure 15. Examples of sprayed films.

Finally, the 48th dispersion seemed to form a promising coating. This coating dispersion was homogeneous, stable and easy to spray. The casted film showed pH-sensitive behavior. It was stable during 2 hours' incubation in HCl medium and it disintegrated in phosphate buffer. The coating formulation consisted of lipid mixture, emulsifier, film forming polymer and plasticizer (see Table 11). All the substances used were non-toxic, of natural origin and to be found both in food and in nutraceutics.

Table 11. Composition of 48th coating dispersion.

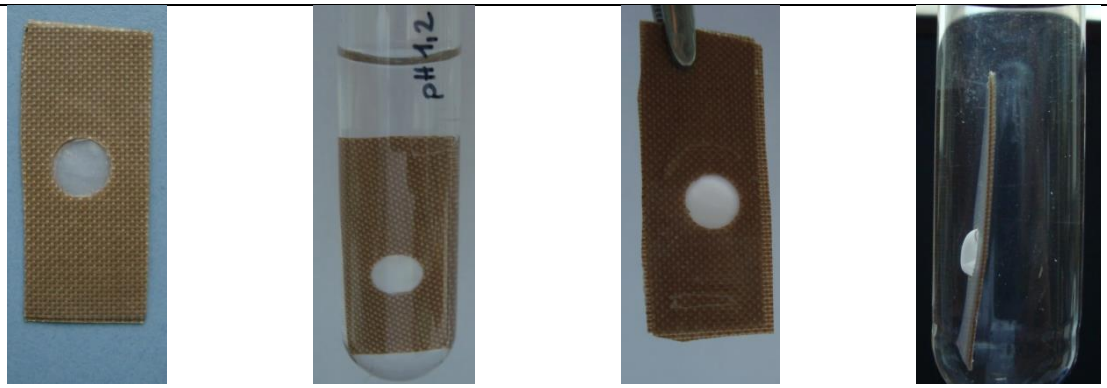
Composition of 48th coating dispersion	Content (%)
HPC	0,8
Water	93,2
OSA Starch	0,5
Glycerol	1,1
Palmitic Acid	1,1
Mono- and diglycerides esterified with citric acid	3,3

3.1.5. Analyses of sprayed films

A sprayed film was first analyzed in disintegration tests. If the film demonstrated fast disintegration (during incubation in acidic medium), the composition of the coating dispersion was modified again. In the case of the lack of disintegration of an investigated film in HCl medium, it was next subjected to water resistance tests. Films showing good water resistance were further examined by differential scanning calorimetry (DSC). Insufficient water resistance characteristic of a film required additional modifications of the composition of coating dispersion.

3.1.5.1. Disintegration tests of sprayed films

The behavior of the 48th film formulation during the disintegration test is presented in Figure 16.



Piece of sprayed film. | 2 h incubation in HCl medium (pH 1.2, 37 ± 0.5°C). | Film transfer from HCl medium (pH 1.2) to phosphate buffer (pH 6.8) after 2 h incubation. | Incubation in phosphate buffer (pH 6.8, 37 ± 0.5°C).

Figure 16. Behavior of the 48th film formulation during disintegration test.

The film presented in Figure 16 showed very high stability. During 2 hours' incubation in acidic medium (pH 1.2) the film remained intact. After 7 hours' incubation in phosphate buffer (pH 6.8) the film became deformed but did not disintegrate. The disintegration occurred after the next 3 hours of the film incubation.

3.1.5.2. Water resistance tests

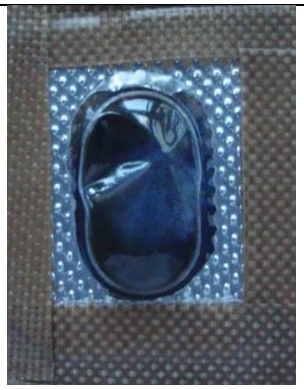
Water resistance tests involved: water resistance test in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8), water resistance test in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (without pancreatin, pH 6.8) and water resistance test in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (with pancreatin, pH 6.8)

Water resistance test in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8)

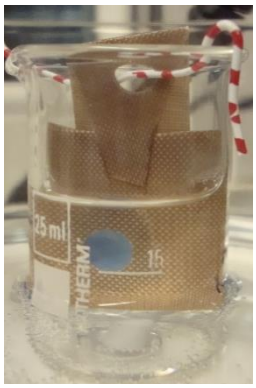
After a film exhibited proper stability confirmed during disintegration tests, its resistance against water was investigated. The performance of the water resistance test of the 48th film formulation is demonstrated in Figure 17.

2th h of film incubation in HCl medium.

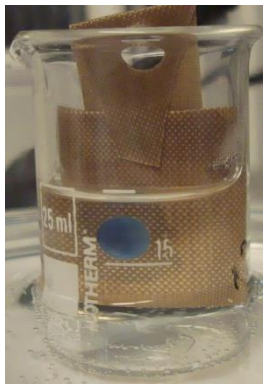
Film after 2 h incubation in HCl medium.



Dry dye 2 h after incubation in HCl medium.



5th h of film incubation in phosphate buffer.



7th h of film incubation in phosphate buffer.

Film after 7 h incubation in phosphate buffer.

Dry dye 7 h after incubation in phosphate buffer.

Figure 17. Water resistance test of the 48th film formulation (in HCl medium and in phosphate buffer).

The 48th film formulation demonstrated very high resistance to water (see Figure 17). The color of the acidic medium did not change during 2 hours incubation. At the same time, the film became slightly blue. However, the dye container remained dry. The color of the phosphate buffer remained unchanged even after 7 hours of the film incubation. Simultaneously, the color of the film was dark blue. Hour after hour, the color of the film became darker, but still no dye release was observed. The further incubation was performed overnight (see Figure 18). After 24 hours the dissolution medium became slightly blue. After the next few hours the color of the medium changed to blue. However, no visible damages of the film could be detected.

Overnight incubation in phosphate buffer (24 h).

Change of the medium color.

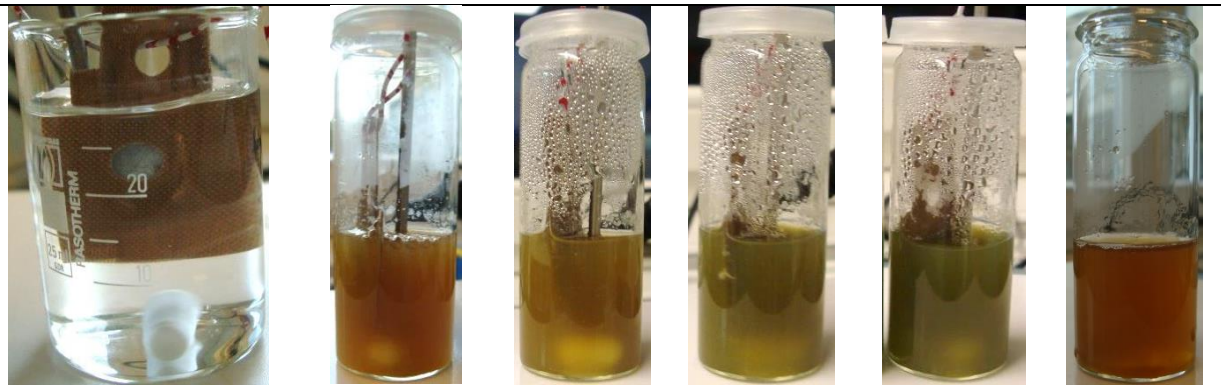
Film after overnight incubation.

Figure 18. Overnight incubation of the 48th film formulation.

Water resistance test in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (without pancreatin, pH 6.8)

The aim of this experiment was to investigate the impact of bile salts and phospholipids on the permeability of the lipid-based coating. Free fatty acids and monoacylglycerols, which are a part of the coating formulation, are capable to interact with bile salts and phospholipids forming mixed micelles [56]. As a result of micellar solubilization the increase of film permeability is possible, thus the release of the dye might be faster (compared to the dye release in phosphate buffer). This test had to demonstrate potential differences in the dye release through the lipid-based film during its incubation in phosphate buffer and in FeSSIF an addition of pancreatin. Any modification of the dye release in comparison to dye release in phosphate buffer will point to the interaction between the tested film and bile salts and phospholipids.

The water resistance test of the 48th film formulation is presented in Figure 19. During 2 hours' incubation of the tested film the color of the HCl medium (pH 1.2) did not change. Also over 4 hours' incubation in FeSSIF without pancreatin (pH 6.8) no change of the medium color was observed. After 4.5 hours the color changed to slightly green. In the 5th hour of the film incubation the medium became greener. After next 30 minutes the medium was green. In order to facilitate the observation of the dye release in tested samples, a sample containing only FeSSIF was simultaneously incubated (blank negative sample).



2nd hour incubation in HCl medium (pH 1.2)	1 h	4 h 30 min	5 h	5 h 30 min	Blank negative

Figure 19. Water resistance test of the 48th film formulation (in HCl medium and FeSSIF without pancreatin).

The results presented above revealed the interaction between the mixture of bile salts and phospholipids (from FeSSIF) and lipids being a part of the coating formulation. The dye release in a presence of bile salts and phospholipids was much faster compared to the dye release in phosphate buffer.

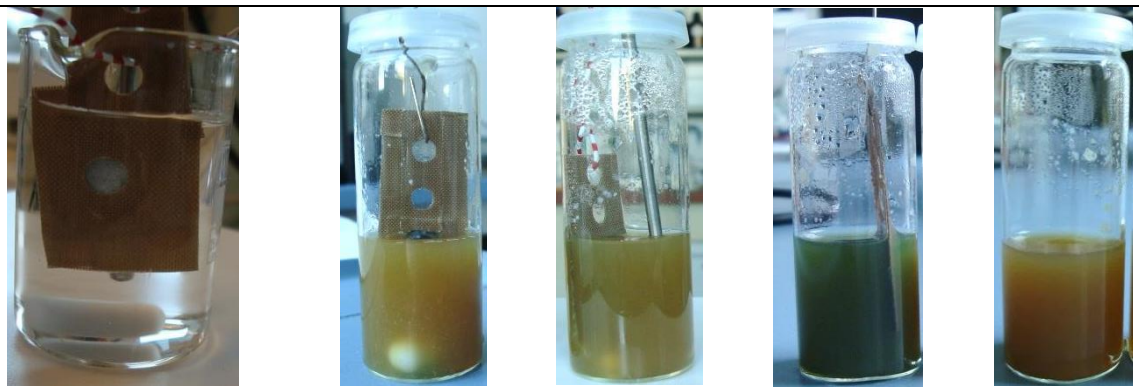
Water resistance test in hydrochloric acid medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (with pancreatin, pH 6.8)

The main components of tested coating formulations constituted lipids. Therefore the fate of lipids within the gastrointestinal (GI) tract should be considered. In the GI tract enzymes responsible for lipids digestion are present. Lipids digestion already begins in the stomach and is caused by gastric lipase. However, the main lipid digestion occurs in the small intestine because of the presence of pancreatic lipase. Pancreatic lipase hydrolyses triacylglycerols first into diacylglycerols and next into monoacylglycerols and free fatty acids [107]. Also the existence of bile in the small intestine is playing a major role in lipids decomposition. Bile contains i.a. bile salts and phospholipids which are able to interact with free fatty acids and monoacylglycerols forming mixed micelles [108]. The release characteristic of API in the GI tract from a dosage form coated with developed lipid-based coating will hence depend on the digestion level of lipids and their interaction with mixed micelles.

Previously performed water resistance tests, using phosphate buffer as a second dissolution medium, can be too inaccurate for the prediction of the active substance release in the human body. The sole factor having impact on the release characteristic in phosphate buffer is the pH value. Only the use of intestinal fluids simulating physiological conditions can provide more accurate release prediction of an active substance in the body. Thus, in the present test as a second dissolution medium a Fed State Simulated Intestinal Fluid (FeSSIF, pH 6.8) was used. It contained pancreatin, bile salts and phospholipids. Pancreatin is a mixture of digestive enzymes including lipase (lipid digestion),

amylase (carbohydrates digestion) and protease (protein digestion). By using FeSSIF in the water resistance tests besides pH value the influence of three more factors, namely the presence of pancreatin, bile salts and phospholipids, on the film permeability was tested.

The water resistance test of the 48th film formulation is shown in Figure 20. The investigated film remained impermeable after 2 hours' incubation in hydrochloric acid medium (pH 1.2). During 3 hours' incubation in FeSSIF (with pancreatin, pH 6.8) the color of the medium did not change and remained yellow. In 4th hour of film incubation the color changed to greenish. After the next 30 minutes the color became significantly greener. For better visual recognition of the methylene blue diffusion through the sprayed film, a blank negative sample (FeSSIF with pancreatin) was simultaneously incubated.



2nd hour of film incubation in HCl medium.	1 h	4 h	4 h 30 min	Blank negative (FeSSIF with pancreatin)

Figure 20. Water resistance test of the 48th film formulation (in HCl medium and FeSSIF with pancreatin).

Comparing the previous experiment performed in phosphate buffer and the current test in FeSSIF (with pancreatin), different release rates of methylene blue were found. The color of phosphate buffer did not change after 7 hours of film incubation, whereas the release of the dye in FeSSIF started already after 4 hours. This indicated that besides pH value other factors had impact on the lipid coating formulation.

The present study revealed also that the lipid-based film did not only interact with bile salts and phospholipids but was simultaneously digested by pancreatic lipase. The highest release rate of the methylene blue was observed in FeSSIF containing pancreatin. The dye release in FeSSIF with pancreatin began one hour faster than in FeSSIF without pancreatin. In conclusion, the water resistance tests indicated the impact of pH value, digestive enzymes, bile salts and phospholipids on the 48th coating formulations. Under their influence the film permeability increased.

3.1.5.3. Differential Scanning Calorimetry analyses

Differential Scanning Calorimetry (DSC) analyses were performed for all solid excipients of the 48th film formulation (see Table 11) and for the film itself.

During the first heating of palmitic acid an endothermic process was detected. At ca. 65°C the lipid melted. The solidifying of palmitic acid occurred at ca. 55°C. During the second heating the sample also melted at ca. 65°C (see Figure 21).

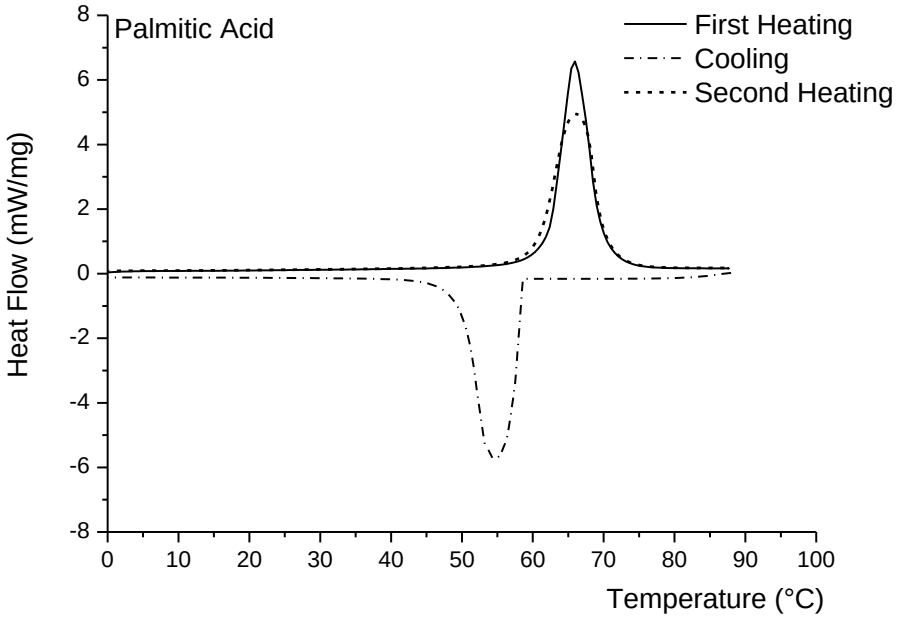
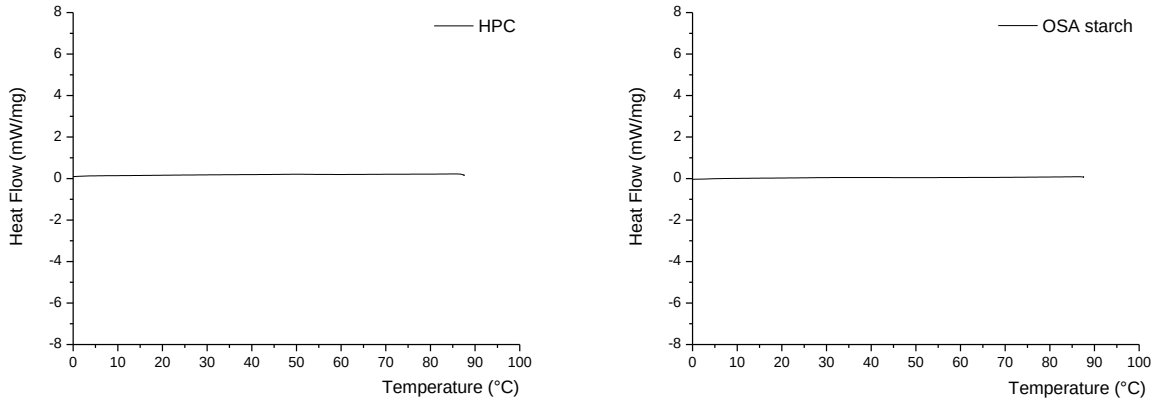


Figure 21. Differential Scanning Calorimetry thermogram of palmitic acid.

HPC and OSA starch showed no physical changes during the DSC analysis (Figure 22).



DSC thermogram of hydroxypropyl cellulose DSC thermogram of OSA starch

Figure 22. Differential Scanning Calorimetry thermogram of hydroxypropyl cellulose (on the left) and of OSA starch (on the right).

During the first heating of citroglycerides, the melting point at ca. 63°C was detected. During cooling two solidification points were present, at ca. 52°C and at ca. 49°C. During the second heating two melting points at ca. 59°C and at ca. 62°C were found (see Figure 23). The appearance of the two solidification and melting points is evidence of the formation of polymorphic forms.

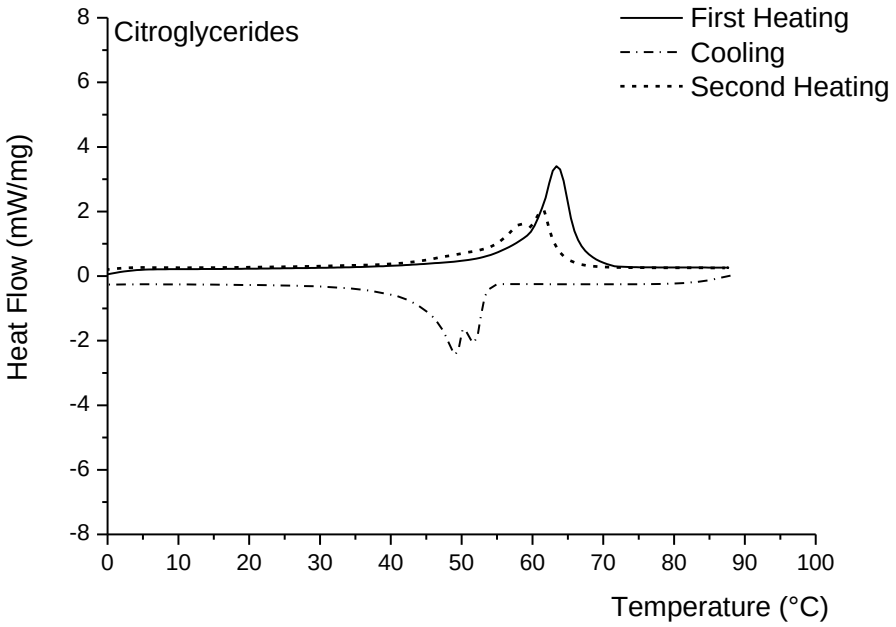


Figure 23. Differential Scanning Calorimetry thermogram of citroglycerides.

The DSC analysis of the 48th film formulation is shown in Figure 24. The first heating detected two melting points, at 52°C and at 58°C. On the cooling curve three solidification points were visible, at ca. 51°C, at ca. 42°C and at ca. 32°C. During the second heating three melting points were observed, at ca. 48°C, at 51°C and at ca. 57°C. The composition of the film and the concentrations of its components affected the temperatures of melting and of solidification of the tested film. A mixture of substances will melt at lower temperatures, compared to melting temperatures of pure substances, as a result of freezing point depression [109]. Also glycerol, used as a plasticizer in the film formulation, resulted in the shift of the melting points to lower temperatures.

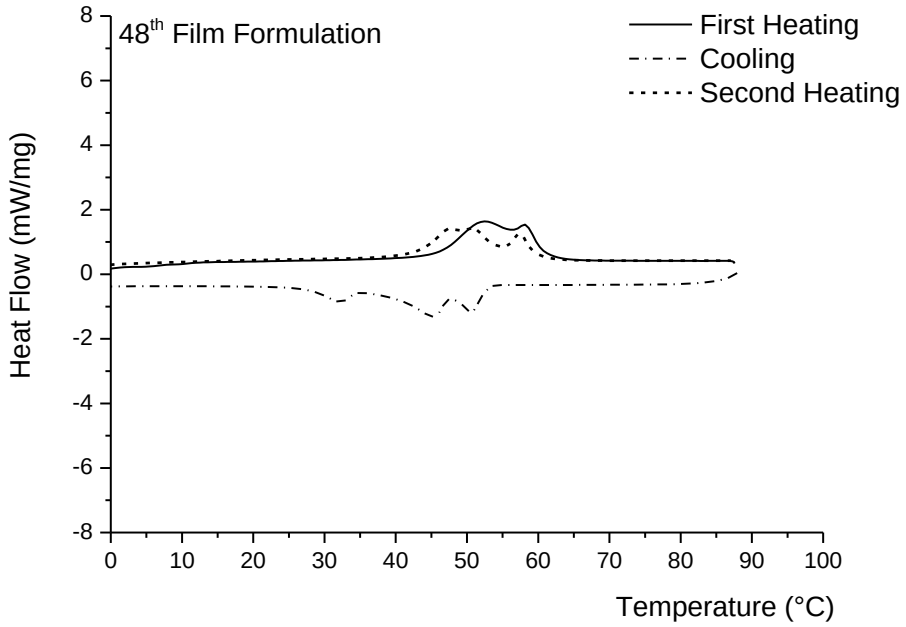


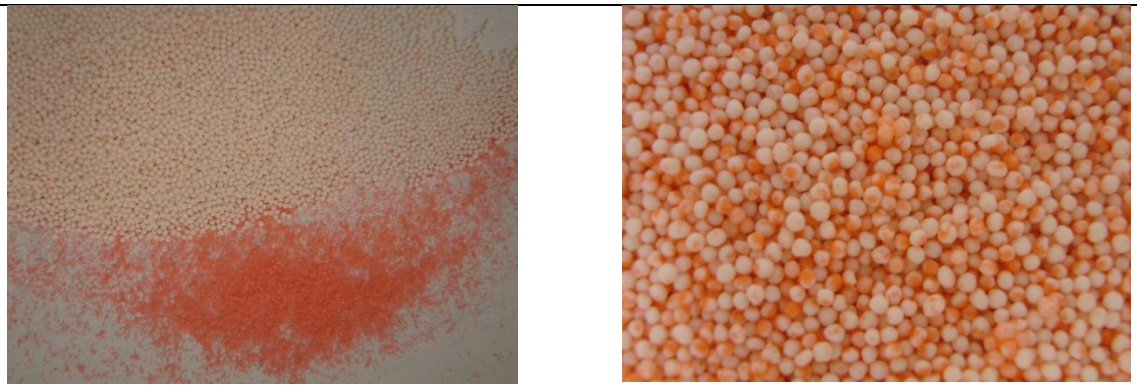
Figure 24. Differential Scanning Calorimetry thermogram of 48th film formulation.

3.1.6. Coating process and further formulation development

Once the coating dispersion showing suitable attributes was obtained, it was next used for the coating of pellets. The coating process was performed in fluidized bed (see Chapter 2.2.5). A crucial step during the coating process was the film formation on pellets surface. The forming film had to show proper adhesion to the cores surface and simultaneously an adequate cohesion between the film molecules. In this manner, a coating characterized by good properties could be created.

The film formation was not only dependent on the dispersion composition but also on the coating parameter settings. Therefore, to ensure proper film formation an adjustment of coating parameters was necessary. During the coating process the following parameters could be adjusted: Atomizing Air Pressure (AAP), Air Flow (AF), Inlet Air Temperature (IAT), Outlet Air Temperature (OAT) and Spray Rate (SR). Through changes in Atomizing Air Pressure the size of dispersion droplets could be adjusted. Dispersions with higher viscosity required higher AAP in order to form finer droplets. A proper value of Air Flow ensured suitable movements of the fluid bed. In some cases the weight gain of pellets during the coating process required an increase of the AF rate. For the film formation a proper product temperature was also essential. The product temperature could be adjusted indirectly, through setting of the Inlet Air Temperature. Too high product temperature led to too rapid drying of the layer and thus to disorder of the film particles coalescence, whereas too low product temperature resulted in the overwetting of pellets, their agglomeration and film deformation. An adequate spray rate prevented the agglomeration of pellets and ensured the formation of homogeneous film.

The first dispersion used for the coating of pellets was the 48th dispersion. It demonstrated good properties including homogeneity, stability and sprayability. Moreover, it was able to form pH-sensitive film with suitable characteristic (confirmed during screenings). Unfortunately, during the coating process the film was not able to hold onto the pellets' surface (see Figure 25). Alterations of the coating parameters did not cause film formation on the particles' surface either. Modifications of the product temperature contributed to covering pellets only partially.



No film formation on pellets surface. Partially covered pellets.

Figure 25. Disturbance of film formation during the coating process. Sudan red dye was used for a better recognition of the coating.

The failure of the coating process with the 48th formulation required further formulation development. In the ideal case, a developed coating dispersion will form a coherent film on pellets surface showing adequate mechanical strength, flexibility and pH-dependent behavior. At the same time, no agglomeration of pellets will occur during the coating process. However, many of the created dispersions demonstrated insufficient properties, which was revealed just during the coating process. Many dispersions were not able to form a film around pellets. In other cases, where dispersion was able to film formation on particles' surface, the agglomeration of the coated pellets constituted a major problem. This required further alterations of the formulation composition as well as adjustment of coating parameters. The selection of proper formulation components and choice of appropriate coating parameter settings were very complex and time-consuming. 18 more formulations were produced after the development of the 48th coating dispersion. In Table 12 the investigated formulations with their characteristics are listed. For some of these formulations several coating processes were performed in order to test the influence of the coating parameters on the film formation. In the column "pH-dependency" the "+" symbol means the pH-sensitive behavior of the coating. In this case the film did not disintegrate during incubation for 2 hours in hydrochloric acid medium (pH 1.2, 37 ± 0.5°C), whereas in phosphate buffer (pH 6.8, 37 ± 0.5°C) the disintegration of the film occurred or the film was deformed. The symbol "-"means insufficient pH-dependency because of fast disintegration of a tested film (independent from pH value). The term

"low mechanical strength" included too brittle or too soft films. The estimation of mechanical strength and flexibility of a film was carried out visually.

The further development of coating formulations was meant to overcome issues associated with the lack of film formation, agglomeration of coated particles, low mechanical strength, insufficient pH-sensitivity, and too fast disintegration of the coating. For this purpose several modifications were performed, which included: reduction of hydrophilic components, change of the film forming polymer, increase of lipophilic pH-dependent excipients, increase of lipophilic pH-independent components, change of plasticizers etc. The major difficulty lay in finding the compromise between the proper mechanical strength of the coating and the formation of the film. Components allowing film formation often caused a formation of too soft films leading to the agglomeration of pellets. However, the reduction of these softening excipients led to the lack of film formation on the pellets surface (Figure 26).

Agglomeration of pellets.

Accumulation of film particles inside the coating device.

Figure 26. Encountered problems during the coating process.

Table 12. List of investigated coating formulations and their characteristic.

No.	Characteristic of produced formulation	pH-dependency
49	No film formation on pellets surface, 4 coatings experiments performed	+
50	No film formation on pellets surface, 3 coatings experiments performed,	-
51	Film formation on pellets surface, very high agglomeration of pellets, very low mechanical strength of formed film	+
52	Film formation on pellets surface, high agglomeration of pellets, low mechanical strength of formed film	-
53	Film did not hold on the pellets surface	-
54	To viscous dispersion, not sprayable	Not applicable
55	Film formation on pellets surface, low agglomeration of pellets, low mechanical strength of formed film	-
56	No film formation on pellets surface	-
57	Film formation on pellets surface, no agglomeration of pellets, low mechanical strength of formed film	-
58	Unstable coating dispersion	Not applicable
59	No film formation on pellets surface, low stability of coating dispersion	Not applicable
60	No film formation on pellets surface, low mechanical strength of casted film	Not applicable
61	No film formation on pellets surface, non-homogeneous casted film	Not applicable
62	Film formation on pellets surface, film with cracks	-
63	Film formation on pellets surface, very high agglomeration of pellets, very low mechanical strength of formed film	-
64	Film formation on pellets surface, no agglomeration of pellets, very low mechanical strength of formed film	-
65	Film formation on pellets surface, no agglomeration of pellets, quite high mechanical strength of formed film	+
66	Film formation on pellets surface, no agglomeration of pellets, high mechanical strength of formed film, 5 coatings experiments performed	+

Finally, the 66th developed coating formulation was able to form a coherent, homogenous film around the pellets. The film had good mechanical strength, was flexible enough and showed pH-dependent behavior. Moreover, during the coating process no agglomeration of pellets was observed. The composition of the 66th coating dispersion is shown in Table 13.

Table 13. The composition of the 66th coating dispersion.

Fromulation 66	[%] in coating
Hydroxypropylcellulose	16,4
Imwitor 372P	61,7
Imwitor 375	5,4
Palmitic acid	16,4

The 66th dispersion was used five times for the coating of pellets in order to optimize the parameters of the coating process. These parameter settings are presented in Table 14. The Air Flow needed to be adjusted during the coating process. Pellets gaining weight required more Air Volume for the right movements allowing proper pellets coating. The most favorable Inlet Air Temperature was 45°C, while the Outlet Air Temperature was 39°C. Higher Inlet Air Temperatures had a negative influence of the film formation, whereas lower temperatures caused agglomeration of pellets. Higher Inlet Air Temperatures were also undesirable, because of the Lower Critical Solution Temperature (LCST) of hydroxypropylcellulose (HPC), which range is ca. 44-46°C [99]. Above the LCST HPC is not soluble. For this reason higher product temperatures will interfere with the coalescence process of film molecules. After the coating of pellets was finished, the Inlet Air Temperature was reduced gradually to room temperature in order to allow the formation of a homogenous and coherent film.

Table 14. Optimized parameters for the coating process.

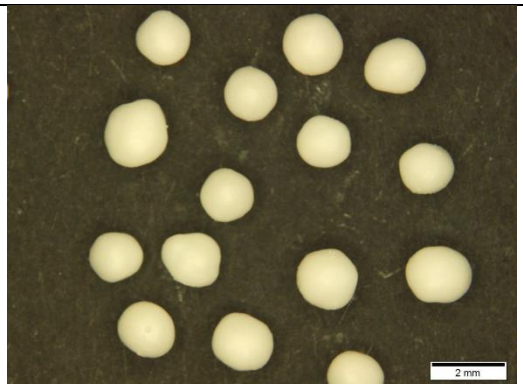
Parameter	Value
Atomizing Air Pressure (Nozzle Air)	14,0 [psi], (ca. 0,97 [bar])
Air Flow (Air Volume)	120-160 [LPM]
Inlet Air Temperature	45 [°C]
Outlet Air Temperature	39 [°C]
Spray Rate (Pump Output)	0,3 [g/min]

3.2. Analysis of the 66th formulation

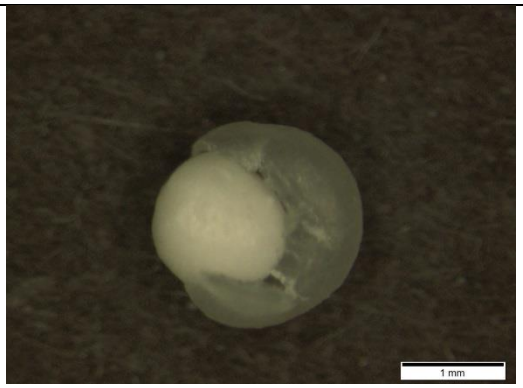
The 66th coating dispersion was the first obtained dispersion with the most satisfactory properties. It was stable and formed a pH-sensitive film around pellets. The analyses of this coating formulation are presented in detailed below and they included: the evaluation of coated pellets under the microscope, pH-dependency tests of coated pellets, water resistance test of sprayed film, film permeability tests, Electron Paramagnetic Resonance studies, and dissolution tests.

3.2.1. Evaluation of coated MCC pellets under the microscope

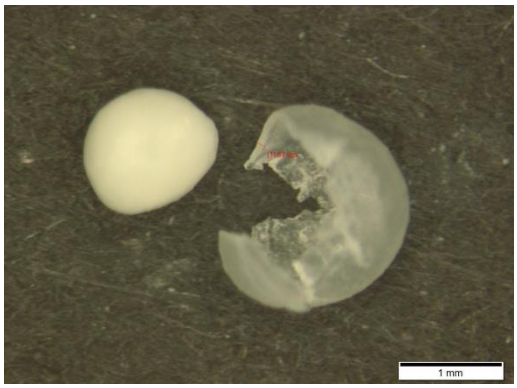
After the coating of placebo microcrystalline cellulose (MCC) pellets with the 66th formulation the pellets were observed under the microscope. The film surface was homogenous, without cracks. The film was coherent and demonstrated good adherence to the particles surface. It had good mechanical properties. The film was not brittle, not too soft and it showed enough flexibility. It was not possible to remove it easily from the pellets surface. During the straightening the film demonstrated good flexibility and good mechanical strength (Figure 27).



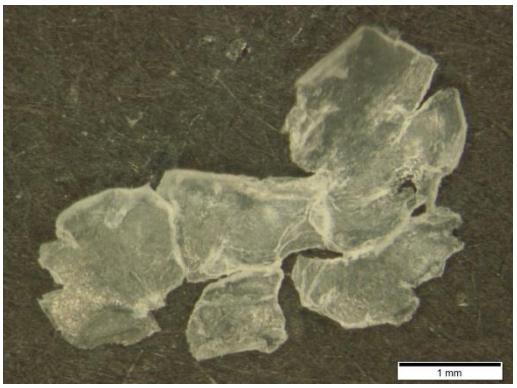
Coated pellets.



Shell around the pellet.



Removal of the pellet shell (shell thickness ca. 87 μm).

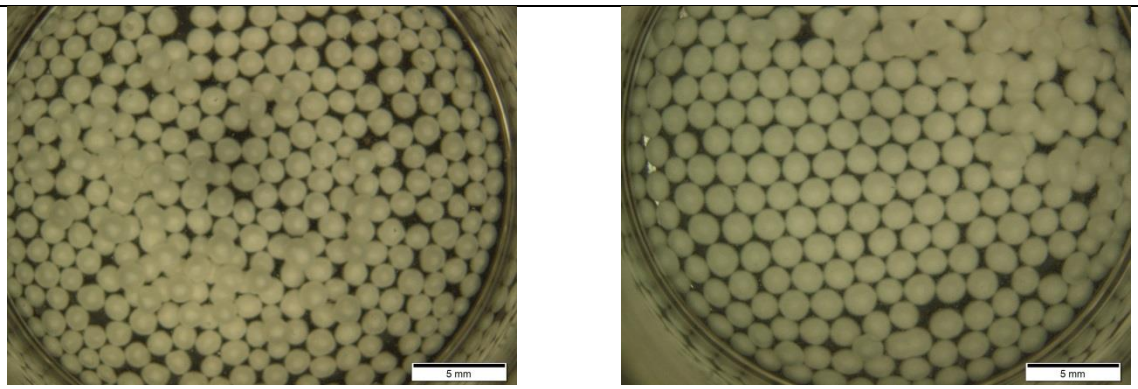


Straightened film.

Figure 27. Observation of placebo MCC pellets coated with 66th dispersion under a microscope.

3.2.2. PH-dependency test of coated pellets

After 2 hours' incubation in hydrochloric acid medium (pH 1.2), the pellets did not change their appearance. They were not "swollen", the film was smooth, without cracks. In contrast, pellets after 2 hours' incubation in phosphate buffer (pH 6.8) looked "swollen", nonetheless without cracks (Figure 28).

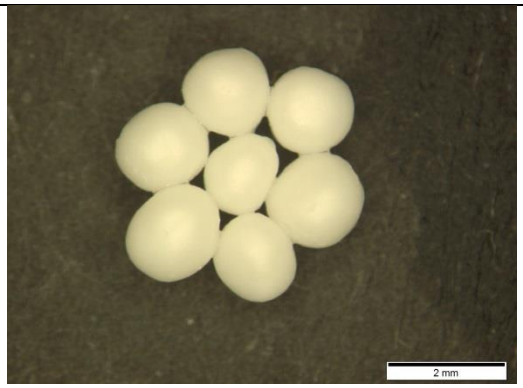


Pellets after 2 hours incubation in HCl medium (pH 1.2).

Pellets after 2 hours incubation in phosphate buffer (pH 6.8).

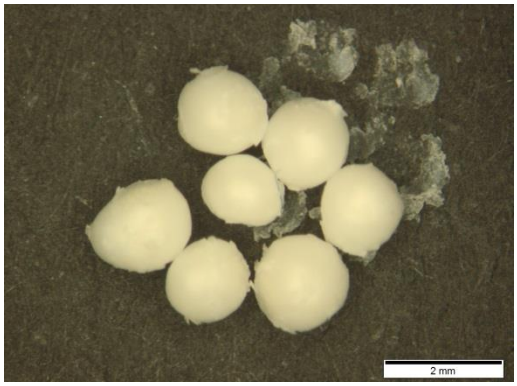
Figure 28. Pellets coated with 66th dispersion after 2 hours' incubation at room temperature in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8).

Subsequently, the pellets were taken from the dissolution media and subjected to mechanical stress. The film on the pellets surface after 2 hours' incubation in pH 1.2 showed much higher mechanical strength compared to the film incubated in phosphate buffer (pH 6.8). Only the outer shell of the film could be removed, whereas the inner shell remained intact. On the other hand, the film on pellets incubated in phosphate buffer (pH 6.8) was very easy to efface. It was of cream-like consistency (Figure 29).



Pellets taken out from HCl medium (pH 1.2) after 2 hours' incubation.

Pellets taken out from phosphate buffer (pH 6.8) after 2 hours' incubation.



Pellets from HCl medium (pH 1.2) subjected to mechanical stress.

Pellets from phosphate buffer (pH 6.8) subjected to mechanical stress.

Figure 29. Pellets coated with 66th dispersion after 2 hours' incubation in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8) subjected to mechanical stress.

3.2.3. Water resistance test of sprayed film

The water resistance test of the 66th formulation was performed in acidic medium (pH 1.2) and in Fed State Simulated Intestinal Fluid (with pancreatin, pH 6.8). First, the 66th coating dispersion was prepared and next sprayed on a polytetrafluoroethylene (PTFE) surface fixed using a spraying device (see Figure 5). Subsequently, the sprayed film's resistance to water was tested. During 2 hours' incubation in hydrochloric acid medium (pH 1.2, 37 ± 0.5°C) the dye remained dry and no change of the medium color was observed (Figure 30). After the medium change to FeSSIF (pH 6.8, 37 ± 0.5°C) the blue color of the film became more intense and the film surface began to be deformed. After 5 hours' incubation the film started to stratify but the FeSSIF remained still yellow. After next 30 minutes the medium was greenish. After next 15 minutes the film broke and the color of the dissolution medium became green (Figure 31).

Film before incubation in HCl medium.

2 h incubation in HCl medium.

Film after 2 h incubation in HCl medium.

Dry dye container after 2 h incubation in HCl medium.

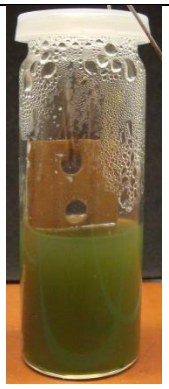
Figure 30. 2 hours' incubation of sprayed film in hydrochloric acid medium (pH 1.2, 37 ± 0.5°C).

Medium change to FeSSIF (t_0).

4 h incubation in FeSSIF.

5 h incubation in FeSSIF.

5 h 30 min incubation in FeSSIF.



5 h 45 min incubation in FeSSIF.

Figure 31. Incubation of sprayed film in FeSSIF (pH 6.8, 37 ± 0.5°C).

3.2.4. Film permeability tests

At first the behavior of the uncoated methylene blue-microcrystalline cellulose (MCC) pellets in dissolution media was investigated. The pellets were placed in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8). The temperature of the media was maintained at 37 ± 0.5°C. Subsequently, the velocity of the color change of the dissolution media was observed (Figure 32).

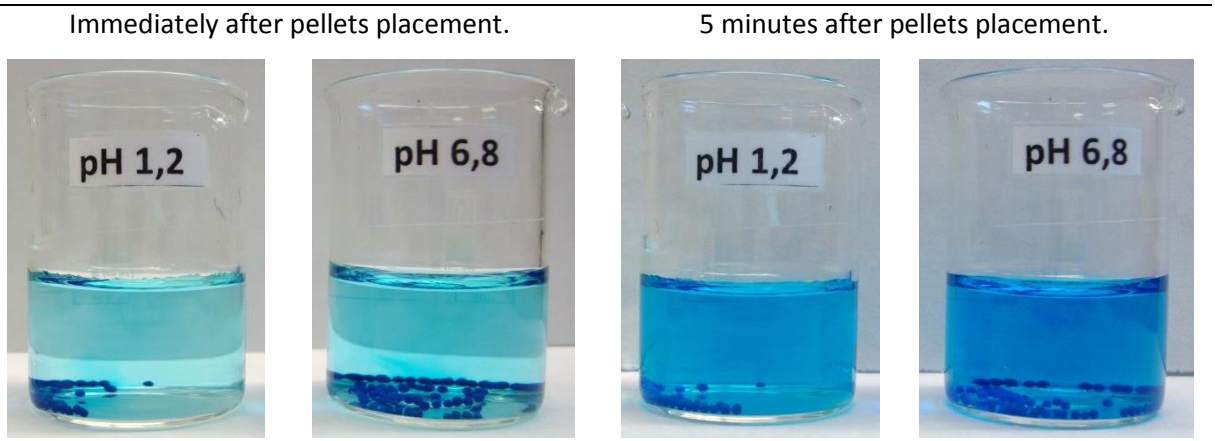


Figure 32. Observation of the color change of dissolution media after the placement of methylene blue-MCC pellets.

The change of the media color to blue was an evidence of the dye release from pellets. The release of methylene blue from was very fast both in HCl medium and in phosphate buffer. Immediately after pellets placement the color of both dissolution media changed to blue. After only few minutes the color was maximally saturated. This experiment demonstrated that the methylene blue-MCC pellets are suitable for release tests using these both dissolution media. The fast and pH-independent release of the dye from pellets will allow a visual evaluation of the coating permeability.

The methylene blue-MCC pellets were then coated with the 66th formulation. Afterwards the permeability of the coating was investigated. Hydrochloric acid medium was used as the first dissolution medium. As the second dissolution media phosphate buffer, Fed State Simulated Intestinal Fluid without pancreatin and Fed State Simulated Intestinal Fluid with pancreatin were used. It was expected, that the developed formulation will show differences in the methylene blue release depending on pH value. For a better recognition of the differences in the dye release in used dissolution media, “blank positive” samples were simultaneously incubated with tested samples. As “blank positive” samples uncoated methylene blue-MCC pellets were utilized.

Film permeability test of coated methylene blue-microcrystalline cellulose pellets in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8)

The color of hydrochloric acid medium (pH 1.2) became slightly blue after 4 hours' incubation of coated methylene blue-MCC pellets. At the same time, the color of pellets turned into light blue (Figure 33). After 5 hours the color of the HCl medium became only little more intense compared to the previous hour. The color of the acidic medium was significantly more intense in 10^{th} hour of the test. However, the blue color of the blank positive samples was still more saturated.

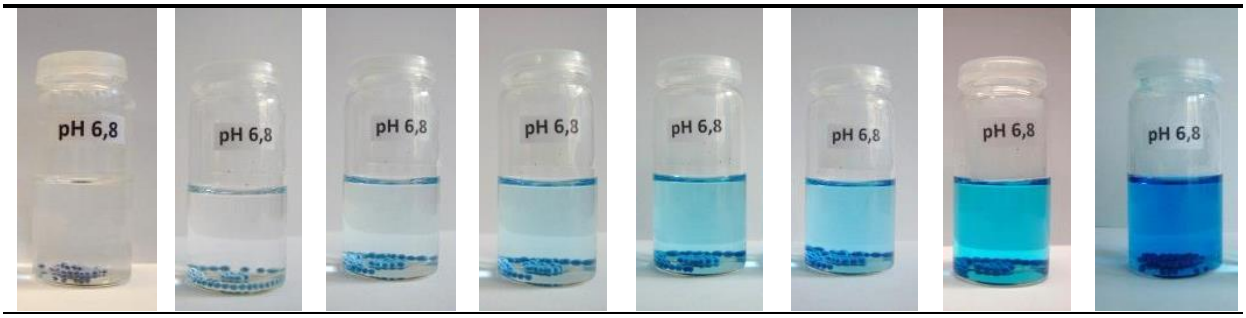
The color of phosphate buffer (pH 6.8) started to change after 2 hours of pellets incubation. Nevertheless, the color of pellets changed to blue already after 1 hour of their incubation. Hour by hour the color of the phosphate buffer and of pellets was becoming more intense (Figure 33).

The change of the medium color can be explained as follows: water was penetrating through the film into the pellet cores. There the dissolution of the dye occurred. Subsequently, the dissolved methylene blue was diffusing through the film to the dissolution medium. This phenomenon was significantly faster in phosphate buffer than in acidic medium.

Hydrochloric acid medium (pH 1.2)

0h | 1h | 2h | 3h | 4h | 5h | 10h | Blank positive

Phosphate buffer (pH 6.8)



0h | 1h | 2h | 3h | 4h | 5h | 10h | Blank positive

Figure 33. Film permeability test of coated methylene blue-microcrystalline cellulose pellets in hydrochloric acid medium (pH 1.2) and in phosphate buffer (pH 6.8).

Analyzing above presented results, a modified, pH- and time-dependent release of the dye methylene blue from coated pellets could be observed. The permeability of the film increased in time and was different in HCl medium (pH 1.2) and in phosphate buffer (pH 6.8). The color of hydrochloric acid medium had changed 2 hours later than the color of phosphate buffer. Within 3 hours of pellet incubation in acidic medium no dye release was observed. In contrast, the release of the methylene blue in phosphate buffer began already after ca. 2 hours of pellets incubation.

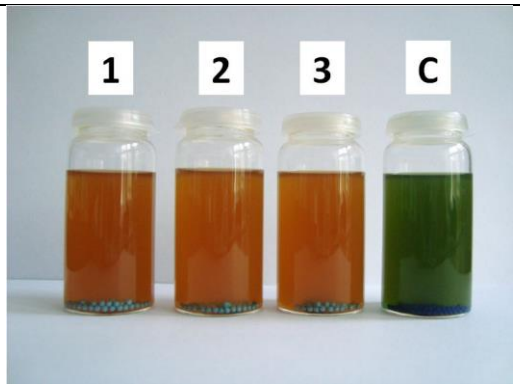
This test gave the first idea about the release characteristic from pellets coated with the developed coating formulation. However, it is insufficient for the prediction of the API release in the human body. Therefore, in the next studies as a second dissolution FeSSIF media were used. FeSSIF by simulating physiological conditions will give more accurate release characteristic of the active substance.

Film permeability test of coated methylene blue-microcrystalline cellulose pellets in HCl medium and in Fed State Simulated Intestinal Fluid (pH 6.8) without addition of pancreatin

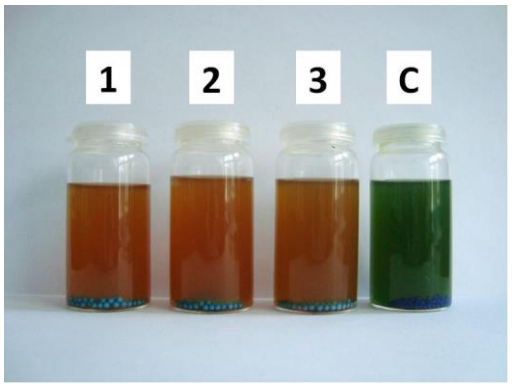
In the present study as the second dissolution medium FeSSIF without an addition of pancreatin was used. This test examined an impact of pH value, bile salts and phospholipids on the developed coating.

The film permeability test of methylene blue-MCC pellets coated with the 66th formulation in FeSSIF without an addition of pancreatin is shown in Figure 34. Figure 34a demonstrated a sample of coated pellets after 2 hours' incubation in hydrochloric acid medium (pH 1.2, 37 ± 0.5°C). Figure 34b-f showed samples incubated in FeSSIF without pancreatin (pH 6.8, 37 ± 0.5°C). In the three first vials (sample 1, sample 2 and sample 3) coated methylene blue-MCC pellets were placed. In the last vial uncoated pellets (blank positive sample - sample C) were incubated.

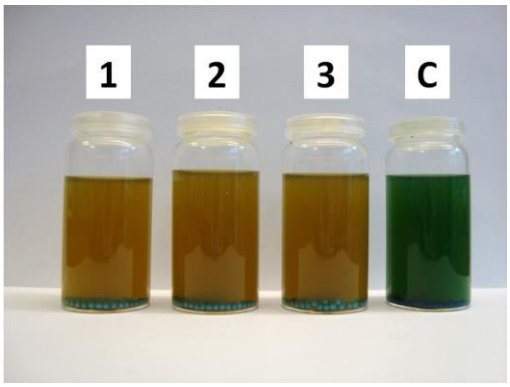
a. 2 h, HCl medium



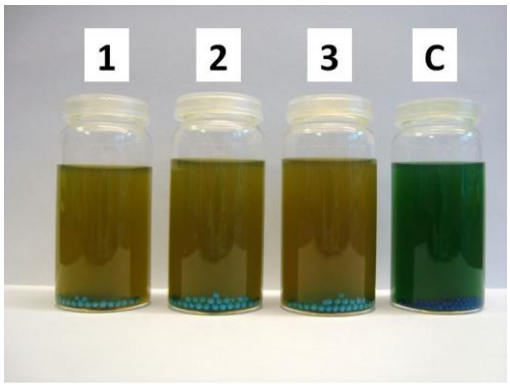
b. 1 h, FeSSIF (without pancreatin)



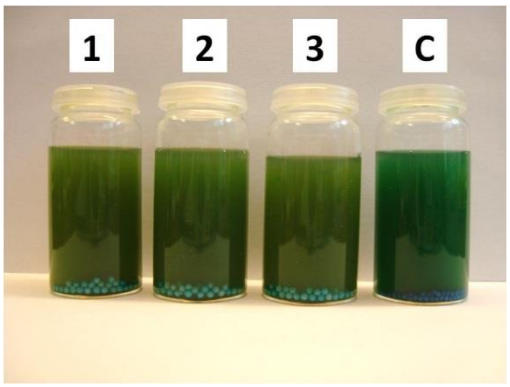
c. 2 h, FeSSIF (without pancreatin)



d. 3 h, FeSSIF (without pancreatin)



e. 4 h, FeSSIF (without pancreatin)



f. 24 h, FeSSIF (without pancreatin)

Figure 34. Film permeability test of methylene blue-MCC pellets coated with the 66th formulation in HCl medium and in FeSSIF without an addition of pancreatin.

During 2 hours of pellets incubation in acidic medium no release of methylene blue was noticed. Over 2 hours also no color change of FeSSIF (without pancreatin) was found. After 3 hours the dissolution medium became green-yellow. One hour later the color was more intense. After 24 hours' incubation the medium color of all samples was quite similar to the medium color of the blank positive sample (sample C).

The comparison of the results of the current and the next study will reveal potential differences in the release rate of methylene blue from coated pellets incubated in FeSSIF without an addition of pancreatin and in FeSSIF with pancreatin.

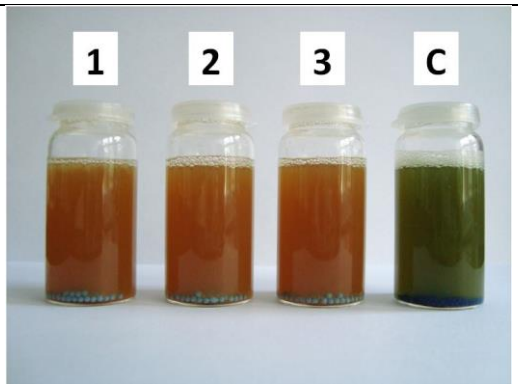
Film permeability test of coated methylene blue-microcrystalline cellulose pellets in HCl medium and in Fed State Simulated Intestinal Fluid (pH 6.8) with pancreatin

Dominant substances constituting the 66th coating formulation are lipids. The main digestion of lipids occurs in the small intestine, because of the presence of pancreatic lipase. Therefore for better prediction of the coating permeability in a human gastrointestinal tract tests in simulated intestinal fluid besides tests in phosphate buffer were performed. For this experiment Fed State Simulated Intestinal Fluid (FeSSIF, pH 6.8) was used. It contains bile salts, phospholipids and pancreatin, which is a source of digestive enzymes (pancreatic lipase, esterases, proteases, amylases).

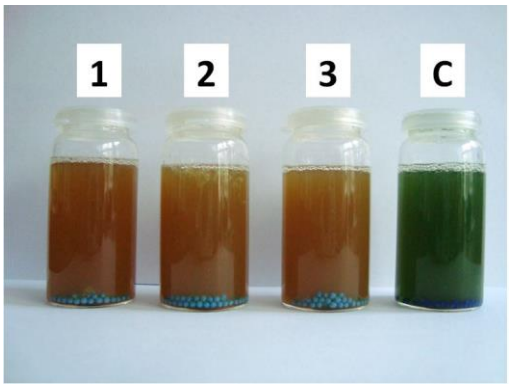
In Figure 35 the film permeability test of coated methylene blue-MCC pellets with the 66th formulation is presented. Figure 35a demonstrated a sample after 2 hours' incubation in hydrochloric acid medium (pH 1.2, 2 h, 37 ± 0.5°C). In Figure 35b-h samples incubated in Fed State Simulated Intestinal Fluid (pH 6.8, 37 ± 0.5°C) are shown. The three first vials (sample 1, sample 2 and sample 3) contained coated pellets with the lipid formulation. In the last vial were uncoated methylene blue-MCC pellets (blank positive sample- sample C). A simultaneous incubation of coated and uncoated pellets allowed an observation of the dye release modification through the developed coating.

After 2 hours of pellet incubation in acidic medium no release of the methylene blue was observed. During 1 hour incubation in FeSSIF (with pancreatin) also no color change was noticed. After 2 hours a slightly change of the medium color was observed. After next 30 minutes the color of FeSSIF became yellow-green. In the 3rd hour a green-yellow color could be observed. After 3.5 hours of pellet incubation the color became more greenish. After next 30 minutes a green color of the medium was noticed. The color of the medium after 24 hours' incubation was very similar to the blank positive sample (Figure 35h).

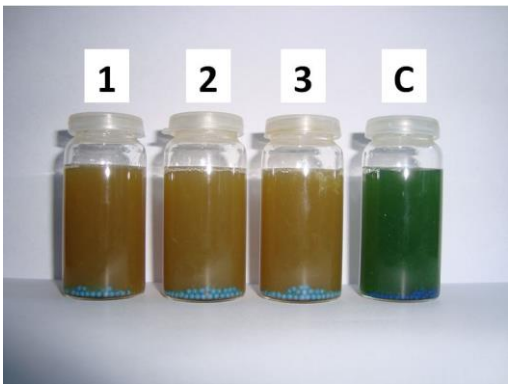
a. 2 h, HCl medium



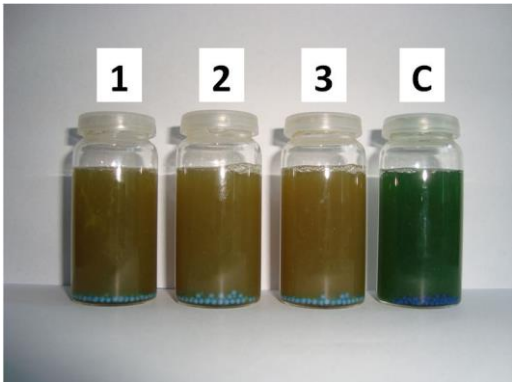
b. 1 h, FeSSIF (with pancreatin)



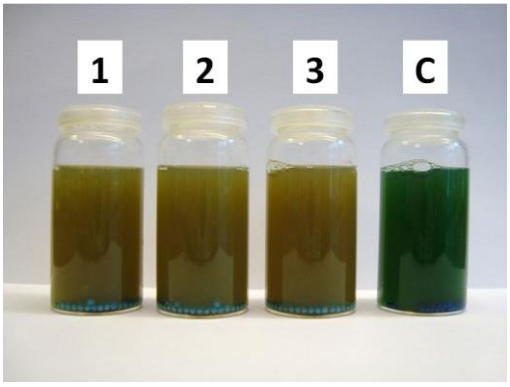
c. 2 h, FeSSIF (with pancreatin)



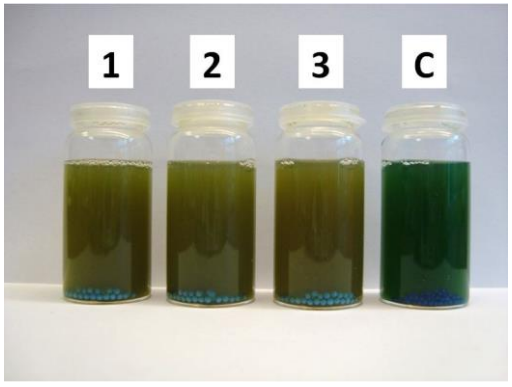
d. 2h 30 min , FeSSIF (with pancreatin)



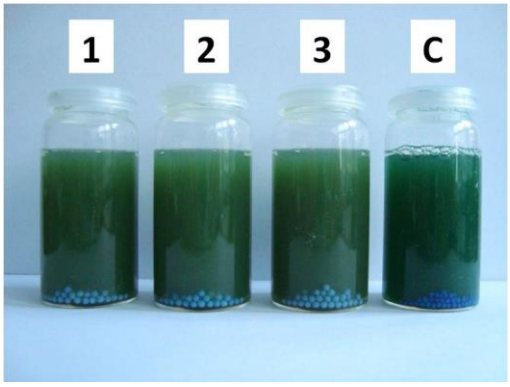
e. 3 h, FeSSIF (with pancreatin)



f. 3.5 h, FeSSIF (with pancreatin)



g. 4 h, FeSSIF (with pancreatin)



h. 24 h, FeSSIF (with pancreatin)

Figure 35. Film permeability test of coated methylene blue-MCC pellets with the 66th formulation in HCl medium and in FeSSIF containing pancreatin; a- 2 h incubation in HCl medium; b-h- incubation in FeSSIF.

Comparing the results of the two last tests, it could be noticed that the color of FeSSIF with pancreatin started to change faster than the color of FeSSIF without pancreatin. The color of FeSSIF with pancreatin became yellow-green already after 2 hours' incubation, whereas FeSSIF without addition of pancreatin had a similar color one hour later. Therefore it can be concluded, that the presence of pancreatin accelerates the release of methylene blue from coated pellets. This indicated the decomposition of lipids being a part of developed coating by digestive enzymes. These tests presented an important idea about the release characteristic from dosage form coated with the 66th formulation in physiological conditions.

3.2.5. Electron paramagnetic resonance studies

Another technique used for the film permeability evaluation was the Electron Paramagnetic Resonance (EPR) spectroscopy, also known as Electron Spin Resonance (ESR) spectroscopy. This method allows the detection of chemical species with at least one unpaired electron (e.g. free radicals, transition metal ions) [110].

In order to use EPR spectroscopy to investigate the coating permeability it was necessary to incorporate a chemical compound with an unpaired electron into pellet cores (see Chapter 2.2.7.). For this purpose 4-hydroxy-tempo (tempol) was used. Tempol is a stable free radical widely used as a spin probe in many experiments [96]. Its structure is presented in Figure 36.

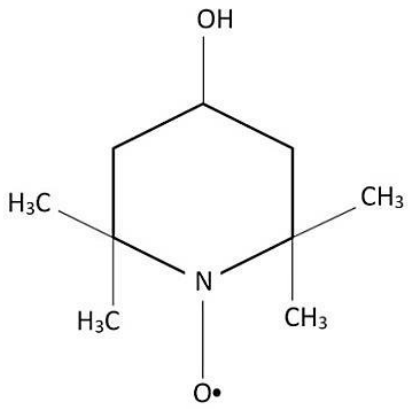


Figure 36. Structure of 4-hydroxy-tempo (tempol).

Pellets containing tempol were coated with the lipid-based coating. The EPR spectrum of coated tempol-microcrystalline cellulose pellets with the 66th formulation is presented in Figure 37.

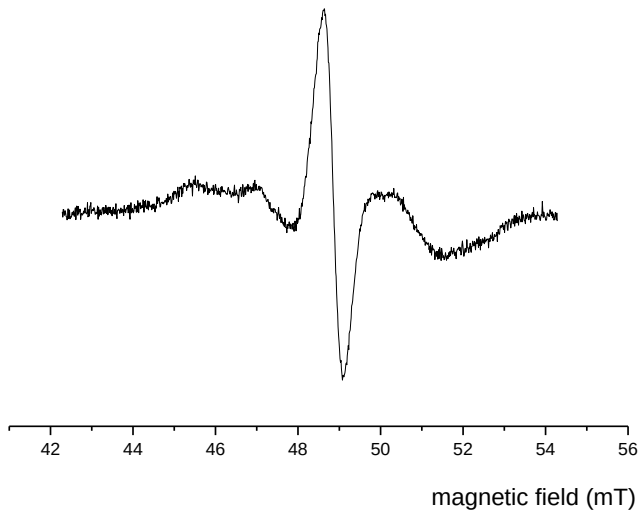
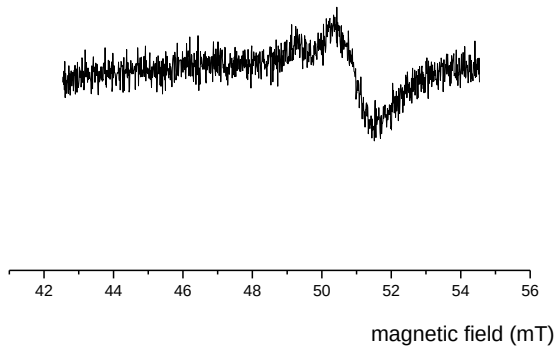


Figure 37. EPR spectrum of coated tempol-microcrystalline cellulose pellets placed in a micro-filter-candle.

The EPR study was only used for a qualitative evaluation of the coating permeability and the model drug release. During the experiment investigated pellets were placed in a micro-filter-candle, which gave EPR signal itself. Even after tempering of this candle filter (560°C, 30 min.) the EPR signal remained (Figure 38). For this reason the EPR measurements were not suitable for a quantitative evaluation. However, this study gave a very important concept about the water movements through the coating. It also revealed differences in tempol release depending on pH value.

Figure 38. EPR signal of micro-filter-candle.



To evaluate the tempol mobility in the sample during the incubation in dissolution media the amplitude changes of the first peak (**1**) and the ratio between the amplitude of the first and of the second peak (**1**:**2**) of the EPR spectra was calculated (Figure 39). The third peak (from the left) was not taken into consideration, because of the possible impact of the signal from the filter candle.

The observation of the amplitude changes of the first peak allowed the evaluation of the water rate influx into the pellet cores and of the diffusion of dissolved tempol via lipid coating to the dissolution medium. The amplitude was growing when the amount of dissolved tempol inside pellet cores was increasing. This was caused by the water influx into pellets. The decrease of the amplitude was caused by the reduction of tempol content in the investigated sample. The dissolved tempol was diffusing through the lipid film from pellet cores to the dissolution medium.

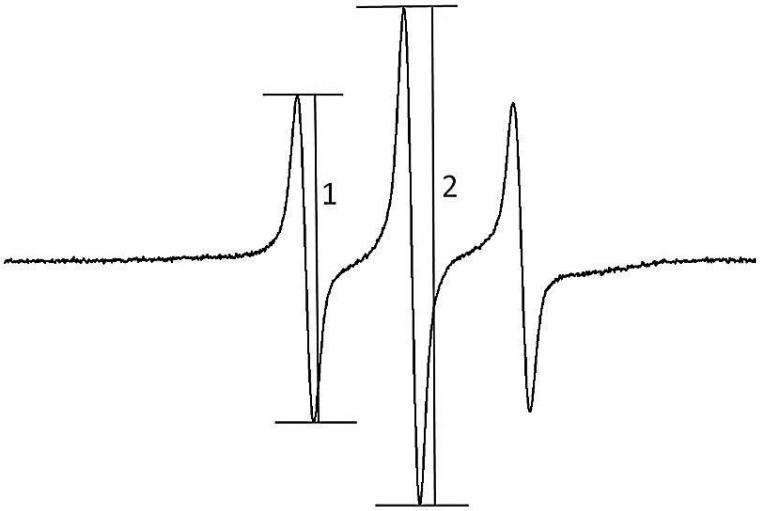


Figure 39. EPR spectrum of lipid coated pellets containing tempol after 0.5 h incubation in phosphate buffer (pH 6.8). The amplitude changes of the first peak (1) and the ratio of the amplitude of the first (1) and of the second (2) peak was used for the tempol mobility evaluation.

The calculation formula of the amplitude ratio between the first and the second peak is presented in Equation 1. The growth of the ratio value indicated the increase of the dissolved fraction of tempol inside pellet cores. The reaching of the ratio value close to 1.0 and the maintenance of a constant level indicated the dissolution of whole content of tempol (the maximal mobility of tempol). As an example an EPR spectrum of dissolved tempol in phosphate buffer (pH 6.8) is presented in Figure 40. Here the values of the amplitudes of the first and of the second signal are very similar. The amplitude ratio of these signals is close to 1.

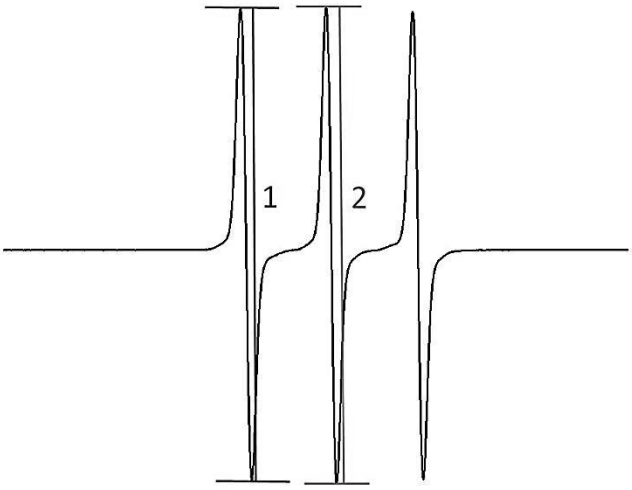


Figure 40. EPR spectrum of tempol dissolved in phosphate buffer (pH 6.8).

$$AR = \frac{\mathbf{1}}{\mathbf{2}}$$

AR - amplitude ratio of first and second peak

1 - amplitude of the first peak (from the left)

2 - amplitude of the second peak (middle peak)

Equation 1. Calculation of amplitude ratio of the first and the second peak.

3.2.5.1. EPR studies of pellets incubated in hydrochloric acid medium (pH 1.2)

EPR spectra of coated tempol-MCC pellets incubated in hydrochloric acid medium (pH 1.2) for 4 hours are presented in Figure 41. The first spectrum presents dry pellets and demonstrates a high immobilization of the spin probe. This spectrum is anisotropic with low amplitudes and broad lines. After 0.5 hour's incubation in acidic medium the EPR spectrum slightly changed compared to that of dry pellets. Next to the middle peak there appeared two others, respectively on its right and left side. However, the amplitude of these two peaks was very small. Over time the peaks were growing. The transformation from anisotropic spectrum of dry pellets to isotropic spectra during incubation indicated the increase of tempol mobility. This was related to the growth of the amount of dissolved tempol inside the pellet cores. Water was penetrating through the film into the pellets, thus the fraction of dissolved tempol was increasing. After 1.5 hours the largest EPR signal could be observed. Since then the signal was decreasing. This indicated the reduction of tempol amount inside the pellets. Tempol was diffusing through the film to the acidic medium. After 4 hours' incubation the EPR signal was barely visible.

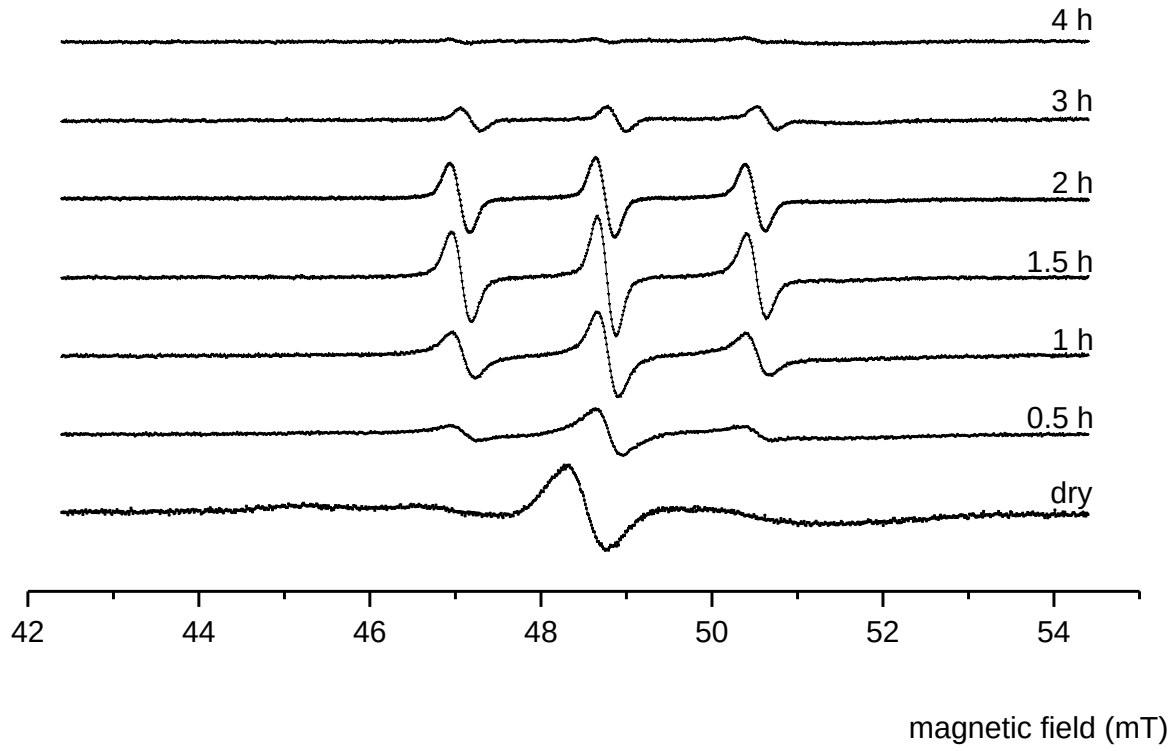


Figure 41. EPR signals of incubated tempol-MCC pellets in HCl medium (pH 1.2) for 4 hours. For comparison the first spectrum presents coated dry pellets before incubation.

The amplitude changes of the first peak (from the left) of the EPR spectrum of coated tempol-MCC pellets during incubation in hydrochloric acid medium are shown in Figure 42. After 0.5 hour's incubation the amplitude increased only slightly. During this time the lipid film showed good barrier against water and the water influx into pellet cores was very low. Afterwards a substantial growth of the amplitude was noticed. The fraction of dissolved tempol was significantly increasing. The maximal amplitude was observed after 1.5 hours of incubation. Subsequently, the amplitude was decreasing. This indicated the decrease of dissolved tempol content in the investigated sample.

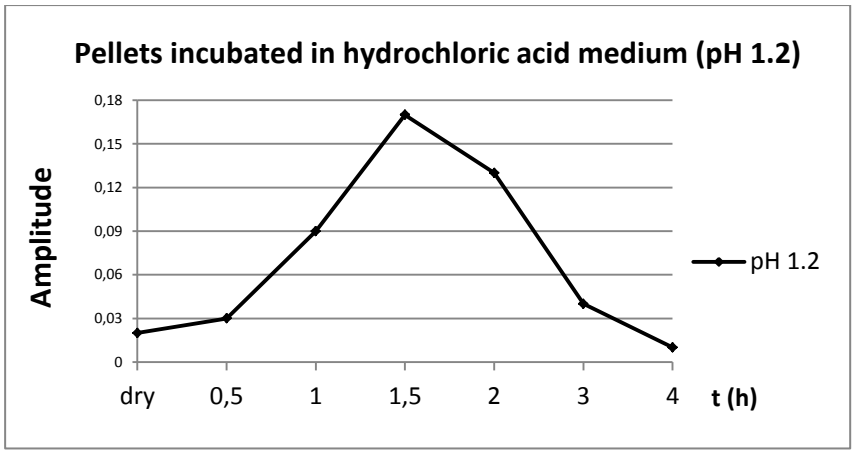


Figure 42. The time dependence of the amplitude of the first signal (from the left) of the EPR spectrum of lipid coated tempol-MCC pellets incubated in hydrochloric acid medium (pH 1.2) over 4 hours.

The graph of the amplitude ratio of the first peak to the second peak of the EPR spectrum of coated tempol-MCC pellets incubated in hydrochloric acid medium (pH 1.2) for 4 hours is presented in Figure 43. The amplitude ratio was growing gradually. The mobility of tempol was increasing over the whole incubation time. The constant ratio value was not reached. This indicated the ongoing dissolution of tempol.

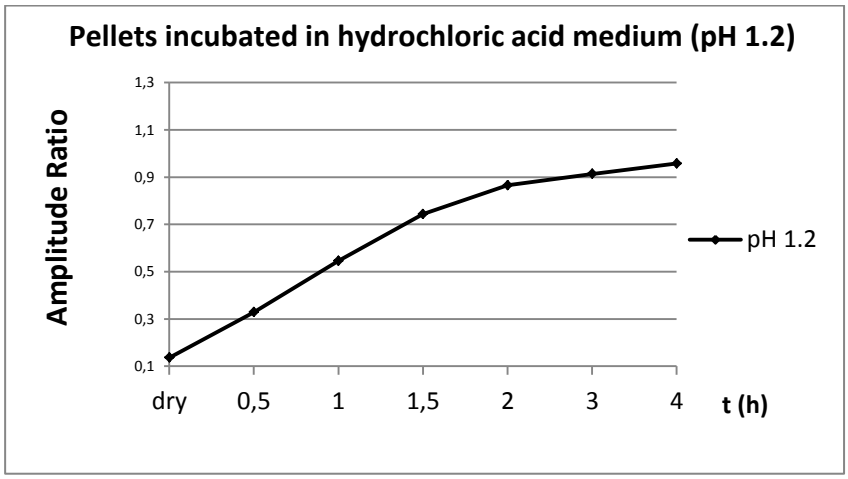


Figure 43. The time dependence of the amplitude ratio of the first and of the second signal of the EPR spectrum of lipid coated tempol-MCC pellets incubated in hydrochloric acid medium (pH 1.2).

3.2.5.2. EPR studies of pellets incubated in phosphate buffer (pH 6.8)

The EPR spectra of tempol-MCC coated pellets incubated for 4 hours in phosphate buffer (pH 6.8) are demonstrated in Figure 44. The first EPR spectrum presents the spectrum of dry lipid coated pellets before incubation. After 0.5 hour of pellets incubation the shape of the EPR spectrum significantly changed compared to the spectrum of dry pellets. Three sharp peaks were already visible. The growing of the peaks indicated the increase of the dissolved fraction of tempol (increase of tempol mobility). This was caused by the water influx into the pellet cores through the lipid film. After next 0.5 hour the signal increased further and was the largest among all signals measured. Since then the EPR signals were significantly decreasing. After 1.5 hours a large fraction of dissolved tempol already diffused through the coating to the dissolution medium. Over time the signals were still decreasing. In the 3rd and 4th hour of sample incubation the signal of tempol was barely identifiable.

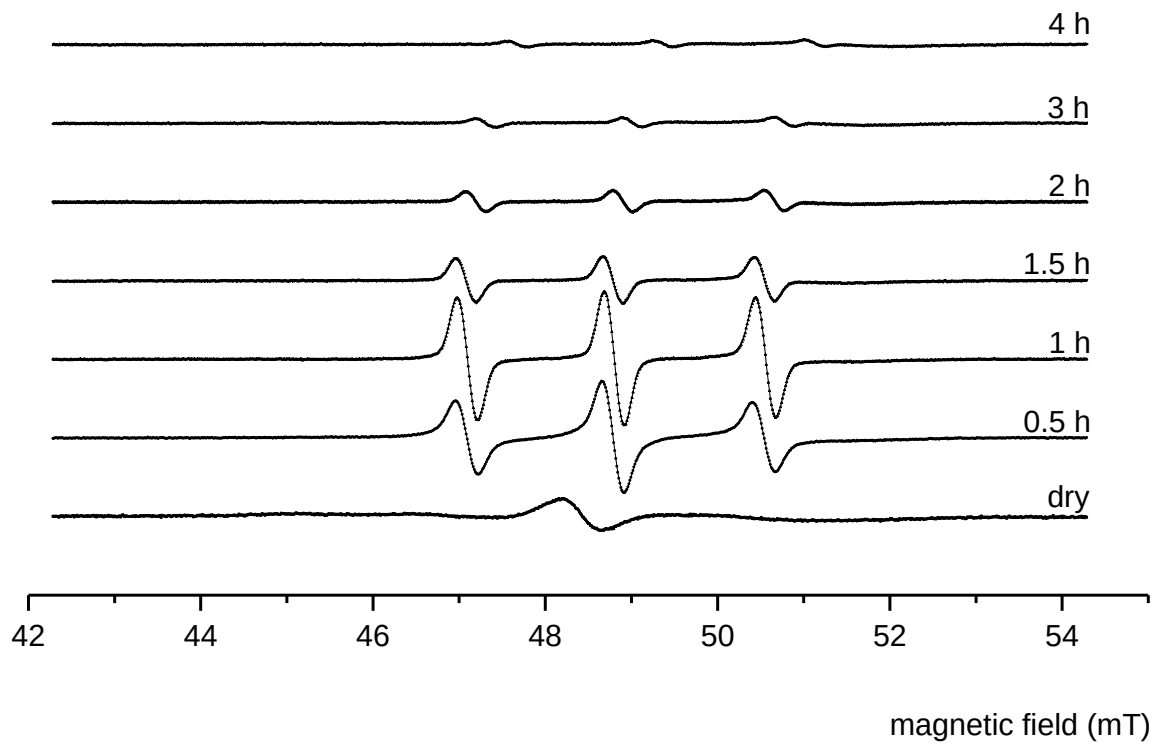


Figure 44. EPR signals of incubated tempol-MCC pellets in phosphate buffer (pH 6.8) over 4 hours. For comparison the first spectrum presents coated dry pellets before incubation.

The amplitude changes of the first peak (from the left) of the EPR spectrum during incubation in phosphate buffer are presented in Figure 45. The amplitude was growing till 1 hour of the pellets' incubation. Water was penetrating through the lipid-based film into the pellet cores. Thus the amount of dissolved tempol inside the pellets was increasing. Subsequently the amplitude of the first signal was significantly decreasing. The dissolved tempol was diffusing to the dissolution medium. In this way the amount of tempol in the investigated sample was decreasing.

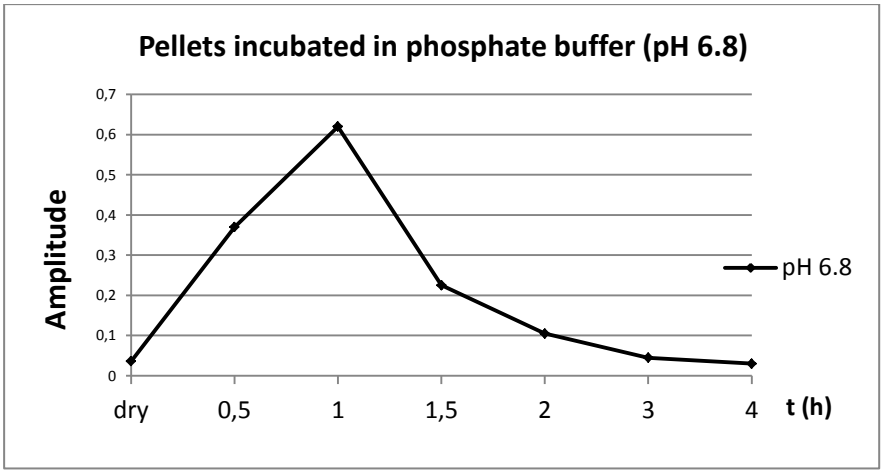


Figure 45. The time dependence of the amplitude of the first signal (from the left) of the EPR spectrum of lipid coated tempol-MCC pellets incubated in phosphate buffer (pH 6.8) for 4 hours.

Figure 46 shows the amplitude ratio of the first peak to the second peak of the EPR spectrum of coated tempol-MCC pellets incubated in phosphate buffer (pH 6.8) for 4 hours. The amplitude ratio was growing fast up to 1 hour. Over the next 0.5 hour the increase of the ratio value was only slight. After 1.5 hours of incubation the amplitude ratio reached a value close to 1.0. Since then the constant level of the amplitude ratio was maintained over the whole measurements duration. The mobility of tempol was maximal.

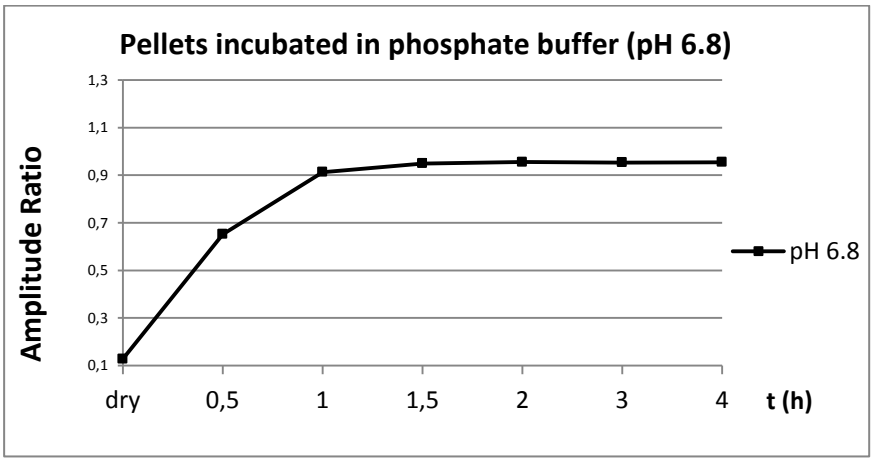


Figure 46. The time dependence of the amplitude ratio of the first to the second signal of the EPR spectrum of lipid coated tempol-MCC pellets incubated in phosphate buffer (pH 6.8).

3.2.5.3. EPR studies of dissolution media

The EPR analysis involved also the dissolution media, in which tempol-loaded pellets were incubated. The detection of EPR signal of tempol indicated the diffusion of the spin probe from coated pellets to the medium.

The results of the EPR analysis of the hydrochloric acid medium (pH 1.2) and of the phosphate buffer (pH 6.8) are shown in Figure 47 and in Figure 48, respectively. The EPR measurements were performed before the placement of coated tempol-MCC pellets in these dissolution media ("0 h") and during their four-hour incubation.

During 4 hours' incubation of lipid coated pellets in HCl medium no EPR signal could be detected. On the other hand, after 1 hour of pellets' incubation in phosphate buffer a spectrum of dissolved tempol could be identified. The EPR signal was continuously increasing with time. This indicated the ongoing diffusion of tempol through the lipid film to the phosphate buffer.

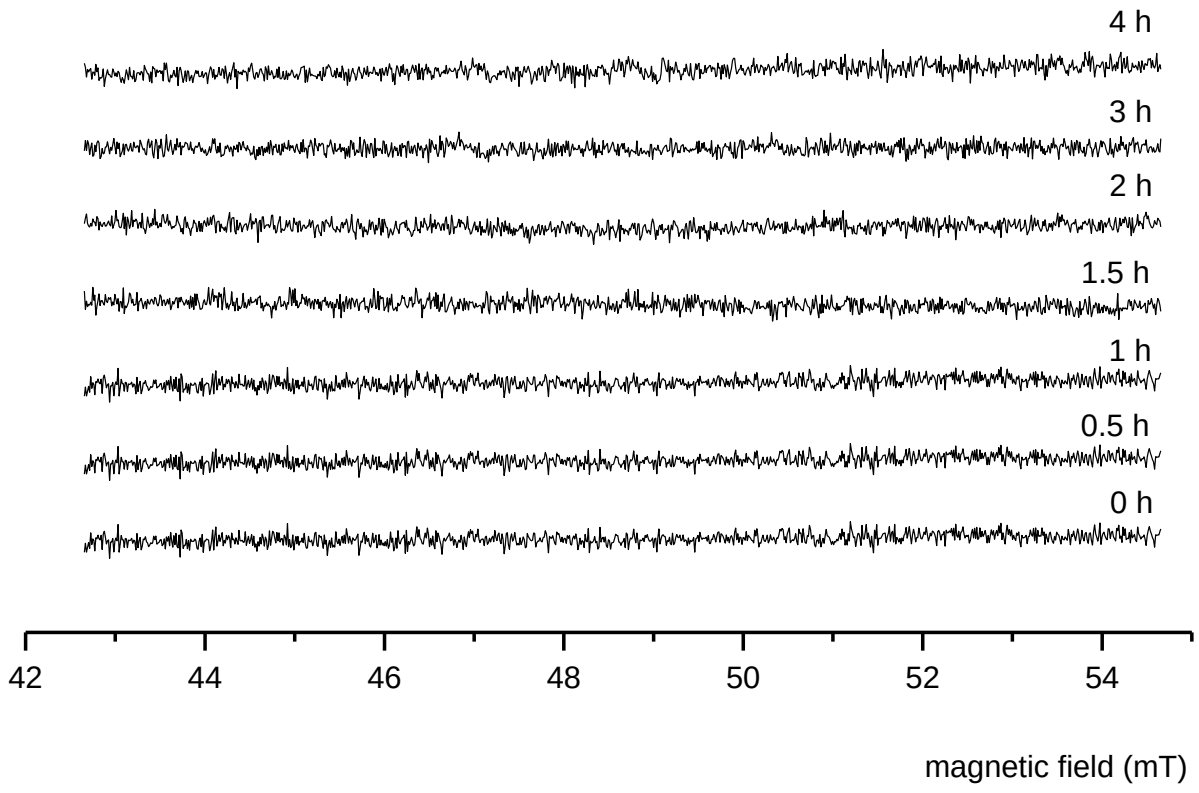


Figure 47. EPR spectra of hydrochloric acid medium (pH 1.2) during 4 hours incubation of investigated lipid coated tempol-MCC pellets. The first measurement was performed before pellets placement in the dissolution medium.

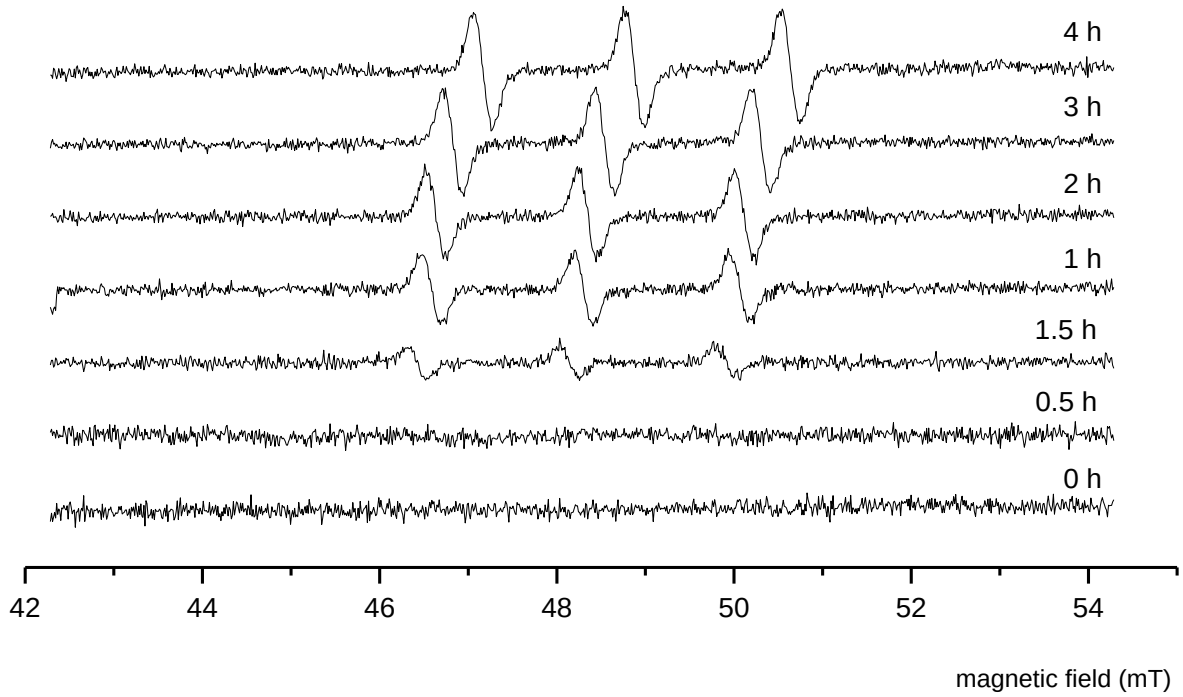


Figure 48. EPR spectra of phosphate buffer (pH 6.8) during 4 hours incubation of investigated lipid coated tempol-MCC pellets. The first measurement was performed before pellets placement in the dissolution medium.

The continuing increase of tempol concentration in the phosphate buffer could be confirmed after the analysis of the amplitude changes of the first peak of the EPR spectrum during pellets' incubation. After 0.5 h the amplitude of the first peak was growing for the entire duration of the test (Figure 49).

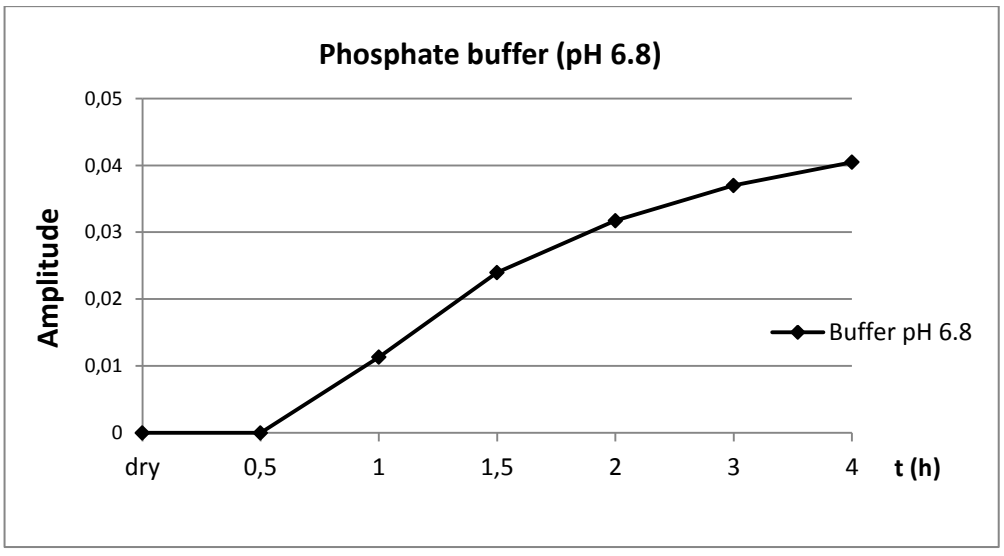


Figure 49. The time dependence of the amplitude of the first peak (from the left) of the EPR spectrum of phosphate buffer (pH 6.8) during incubation of coated tempol-MCC pellets.

The aim of the EPR studies of dissolution media was to reveal the possible differences in tempol release from lipid coated pellets through the measurements of the tempol content in these dissolution media. The results presented above indicated a faster release of the spin probe in phosphate buffer than in HCl medium. However, the whole volume of used dissolution media was taken for the measurements. Because of the electrical conductivity of water it tends to absorb microwaves applied. As a result microwave penetration could be limited and the obtained EPR spectrum could be adulterated [110,111]. Therefore the EPR measurements were used only for a qualitative and rough evaluation.

3.2.5.4. EPR studies-conclusions

The results presented above indicated differences in the velocity of water influx into pellet cores and of tempol diffusion to dissolution media through the lipid coating depending on pH value. The lipid film constituted a greater barrier against water in hydrochloric acid medium (pH 1.2) than in phosphate buffer (pH 6.8). Water influx and tempol diffusion were significantly slower in acidic medium. It can therefore be assumed that the drug release from a dosage form coated with the 66th formulation will be slower in gastric environment (lower pH values) than in intestinal environment (higher pH values).

EPR measurements shed an important light on the release characteristic of pellets coated with the developed lipid-based formulation. However, in order to determine the API release more accurately the performance of dissolution tests with a model drug was necessary.

3.2.6. Dissolution tests

The aim of the dissolution tests was to investigate whether the 66th coating formulation will provide a modified release of the model drug depending on pH value. For the dissolution tests pellets containing caffeine as a model drug were prepared by the saturation technique (see Chapter 2.2.8). Thereafter they were coated with the created coating formulation (coating thickness of 13.9 mg/cm^2). The parameters of the coating process are presented in Chapter 3.1.6.

The release profiles of caffeine from uncoated pellets and from pellets coated with the 66th coating formulation in hydrochloric acid medium (pH 1.2, Figure 50) and in phosphate buffer (pH 6.8, Figure 51) are presented below. The dissolution tests of the coated pellets were performed directly after their coating.

The release of caffeine from the uncoated pellets in hydrochloric acid medium (pH 1.2) was very fast. After one hour the release of API was almost complete. In contrast, the release from lipid coated pellets was visibly delayed. During the first hour only 1.8 % of the drug were released. After following hour 4.5 % of caffeine were detected. After 3 hours the quantity of released API amounted to 12.0 %. Subsequently the release increased gradually (Figure 50).

The release rate of caffeine from uncoated pellets in phosphate buffer (pH 6.8) was the same as in HCl medium. After one hour practically the entire amount of caffeine was released. However the API release from lipid coated pellets in the phosphate buffer differed from the API release in HCl medium. Here, 32.8 % of caffeine were released over the first hour. After 2 hours 82.6 % of caffeine were already detected. The amount of released caffeine after following hour had a value of 94.5 %. Within 4 hours the release was already complete (Figure 51).

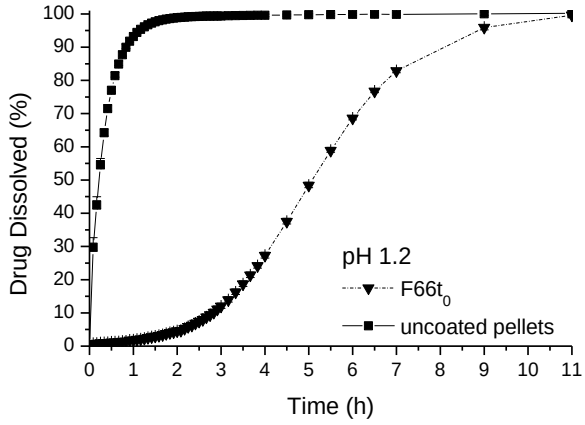


Figure 50. Release profile of caffeine from uncoated pellets and from pellets coated with 66th formulation (F66t$_0$) in hydrochloric acid medium (pH 1.2).

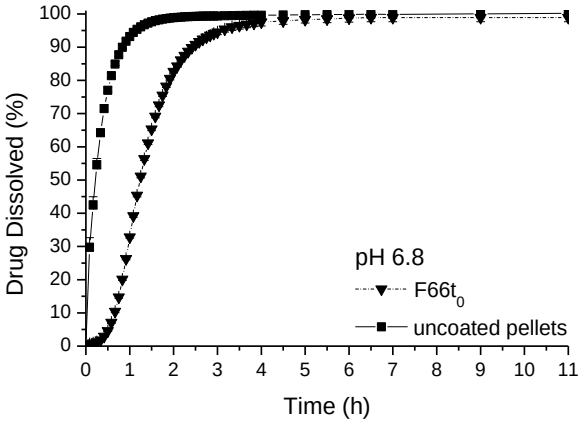


Figure 51. Release profile of caffeine from uncoated pellets and from pellets coated with 66th formulation (F66t$_0$) in phosphate buffer (pH 6.8).

The results of the dissolution tests presented above showed that the developed coating formulation ensured a modified release of the model drug. The release of caffeine was dependent on pH value and it increased with time. In the gastric environment (hydrochloric acid medium) the release of API was delayed. Up to the first 3 hours the release of caffeine was very low (ca. 12 %). The release of API in the intestinal environment (phosphate buffer) was - instead - much faster. Till 3 hours ca. 95 % of caffeine were released. These results indicate that that by means of the created lipid coating a time- and pH-dependent drug release can be achieved. In this manner the aim of this research was achieved.

3.3. Modifications of the 66th coating formulation

The 66th coating formulation was a good starting point for the development of further lipid coatings. Pellets coated with this coating showed an adequate modification of the model drug release. The aim of the additional alterations of the 66th formulation was to obtain a lipid coating ensuring a longer lag phase with minimal API release in the hydrochloric acid medium (pH 1.2), and simultaneously a fast release at higher pH values (phosphate buffer, pH 6.8). At the same time, the developed coating dispersion should be easy to manufacture, possess good characteristics allowing easy coating process and form a suitable film around pellets.

The modifications of the 66th formulation were based only on the change of the citroglycerides type. Citroglycerides constituted the main fraction of the coating formulation. They determined the pH sensitivity of the lipid coating. It was therefore assumed that by using other types of citroglycerides differences in the release characteristics of API will be observed. The amount of all formulation components remained unchanged. This proportion of ingredients ensured the creation of the most favorable formulation (see Table 12). It was therefore expected that due to the use of the same quantity of all excipients good coating dispersions will be formed.

As a result of the modifications of the 66th formulation three other coatings were obtained (67th, 68th and 69th). Their composition is presented in Table 15. The manufacturing procedure was the same for all the coating dispersions (see Chapter 2.2.2).

Table 15. Composition of the 66th, 67th, 68th, and 69th coating formulations.

Fromulation 66	Fromulation 67	Fromulation 68	Fromulation 69	[%] in coating
Hydroxypropyl cellulose	Hydroxypropyl cellulose	Hydroxypropyl cellulose	Hydroxypropyl cellulose	16,4
Imwitor 372P	Imwitor 373 P	Imwitor 370 P	Citrem N	61,7
Imwitor 375	Imwitor 375	Imwitor 375	Imwitor 375	5,4
Palmitic acid	Palmitic acid	Palmitic acid	Palmitic acid	16,4

Subsequently, the new formulations were used for the coating of caffeine-MCC pellets prepared per saturation technique. The coating parameters are given in Chapter 3.1.6.

3.4. Dissolution test of pellets coated with 67th-69th formulation (directly after coating)

The results of dissolution tests of uncoated pellets and of pellets coated with the three new coating dispersions (67, 68, and 69; coating thickness of 13.9 mg/cm^2) are presented below (see Figures 52-57). The test was performed directly after the coating of the pellets. Test conditions were the same for all the formulations and are described in Chapter 2.2.10.6. The uncoated pellets belong to the conventional oral dosage forms showing an immediate release of the active substance. The release of caffeine started just after the pellets' placement in the dissolution medium and was not dependent on the pH value. After one hour the amount of caffeine increased to nearly 100 % both in the hydrochloric acid medium (pH 1.2) and in the phosphate buffer (pH 6.8). The release characteristic of caffeine from the uncoated pellets is presented in all dissolution graphs allowing an easy comparison with the release characteristic of caffeine from lipid coated pellets.

3.4.1. Dissolution tests of 67th coating formulation

Over the first hour of the test in HCl medium (pH 1.2) only 1.6 % of caffeine were released. After 2 hours' incubation the release value increased to 12.4 %. Since then the release was growing significantly. After 3 hours 47.6 % of released caffeine were detected (Figure 52).

The release of the model drug in phosphate buffer (pH 6.8) was much faster compared to the release in HCl medium (pH 1.2). After 1 hour 44.5 % of caffeine were measured. Over the following hour the release increased to 87.4 %. After 3 hours of the test 95.3 % of caffeine were detected (Figure 53).

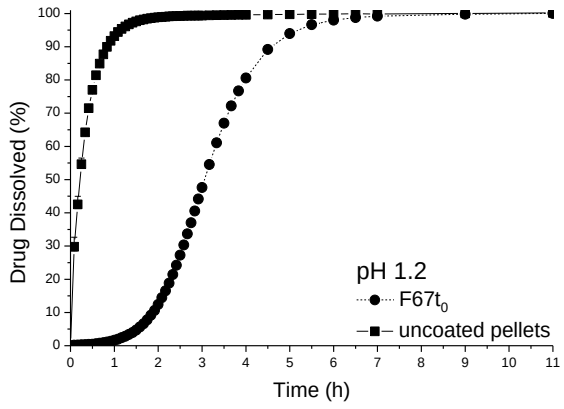


Figure 52. Release profile of caffeine from uncoated pellets and from pellets coated with 67th formulation ($F67t_0$) in hydrochloric acid medium (pH 1.2).

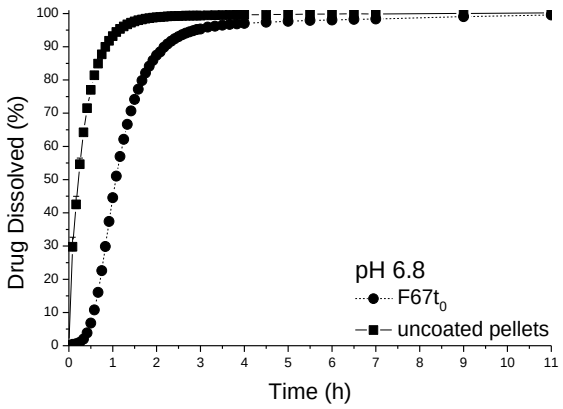


Figure 53. Release profile of caffeine from uncoated pellets and from pellets coated with 67th formulation ($F67t_0$) in phosphate buffer (pH 6.8).

3.4.2. Dissolution tests of 68th coating formulation

The caffeine concentration measured in HCl medium (pH 1.2) after first hour amounted to only 1.3 %. After the following hour it reached 6.4 %. The quantity of caffeine after 3 hours increased up to 20.1 % (Figure 54).

The API content released over the first hour in phosphate buffer (pH 6.8) had a value of 32.6 %. In the 2nd hour of dissolution test it reached 77.7 %. One hour later already 90.5 % were released (Figure 55).

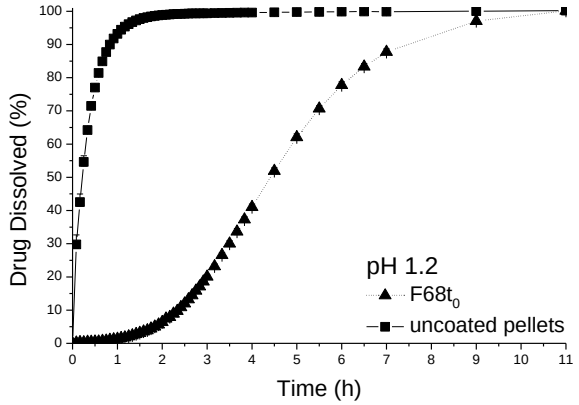


Figure 54. Release profile of caffeine from uncoated pellets and from pellets coated with 68th formulation ($F68t_0$) in hydrochloric acid medium (pH 1.2).

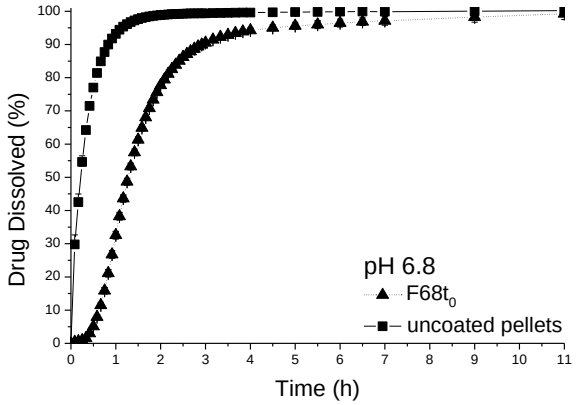


Figure 55. Release profile of caffeine from uncoated pellets and from pellets coated with 68th formulation ($F68t_0$) in phosphate buffer (pH 6.8).

3.4.3. Dissolution tests of 69th coating formulation

The released amount of caffeine in HCl medium after the first hour was barely 0.6 %. One hour later 2.3 % were released. After 3 hours the caffeine content in the dissolution medium increased to only 7.0% (Figure 56).

In the phosphate buffer the measured amount of caffeine after first hour of the test had a value of 20.8 %. After 2 hours 68.3 % of caffeine were detected. Over the following hour the amount of the API reached 86.9 % (Figure 57).

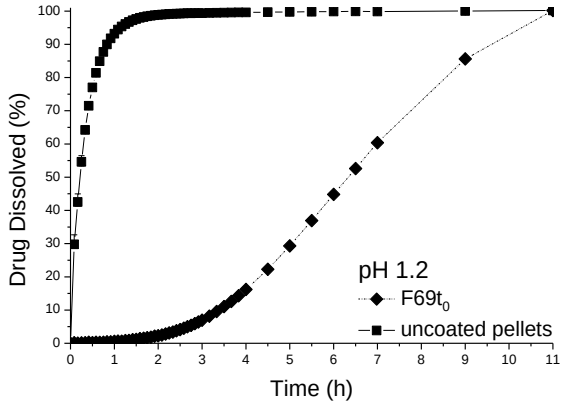


Figure 56. Release profile of caffeine from uncoated pellets and from pellets coated with 69th formulation ($F69t_0$) in hydrochloric acid medium (pH 1.2).

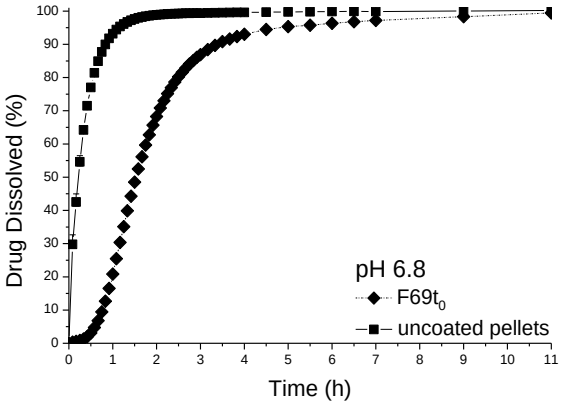


Figure 57. Release profile of caffeine from uncoated pellets and from pellets coated with 69th formulation ($F69t_0$) in phosphate buffer (pH 6.8).

All coated pellets demonstrated a modified release of the active substance compared to the release from uncoated pellets. The release rates of caffeine in HCl medium from the pellets coated with developed formulations was much lower in comparison to the release rate from the uncoated pellets. The release of API from the uncoated pellets started immediately after their placement in the dissolution medium, whereas the delayed release characteristic of caffeine from lipid coated pellets could be observed. During the first two hours the release of caffeine was significantly limited. Subsequently, the release rates as well as the differences between the release profiles of caffeine from the pellets coated with created dispersions were increasing.

The fastest release rate of caffeine in HCl medium was shown by the pellets covered with the 67th coating dispersion. The slowest release rate was exhibited by the 69th formulation. Over the first hour of the dissolution test the amount of released caffeine for all formulations did not exceed 2 % (from 0.6 % for 69th formulation to 1.8 % for 66th formulation). After 2 hours only the 67th formulation released more than 10 %.The other formulations (66th, 68th and 69th) did not release more than 10 %, therefore according to the European Pharmacopoeia they could potentially be used as coatings for gastro-resistant dosage forms [112,113].

In phosphate buffer all the pellets coated with the developed formulations demonstrated significantly higher release rates of the model drug compared to the release rates observed in hydrochloric acid medium. The release of caffeine was delayed in comparison to the caffeine release from uncoated pellets, however. The differences between the release profiles of the investigated formulations in the phosphate buffer were not so significant compared to the differences of the release profiles in the hydrochloric acid medium.

The fastest release of the active substance in phosphate buffer was shown by the 67th formulation, while the slowest was demonstrated by the 69th formulation.

All pellets coated with the lipid-based films released the model drug in a pH- and time-dependent way. The release of caffeine in HCl medium was significantly delayed compared to its release in the phosphate buffer. With time the release of caffeine was also increasing much faster in the phosphate buffer. These results indicated that the developed lipid coatings could potentially be used in delayed release dosage forms. Moreover, three out of four formulations produced (66, 68 and 69) could be applied as gastro-resistant coatings and thereby constitute an alternative for pH-sensitive polymers such as polyacrylates.

3.5. Differential Scanning Calorimetry analyses of 66th-69th formulations

Differential Scanning Calorimetry (DSC) was performed for the uncoated pellets and for the pellets coated with the 66th-69th formulations. For the uncoated pellets no changes in heat capacity were recorded. Changes in heat capacity for the coated pellets are demonstrated below (see Figures 58-61).

3.5.1. Differential Scanning Calorimetry analysis of 66th formulation

After the first heating of the sample one melting point at 58.9°C could be detected. However, two more thermal effects at 50.4°C and at 58.9°C were visible. This could indicate the occurrence of polymorphic forms. During cooling three separate peaks could be observed already, at 51.2°C, at 46.6°C and at 25.3°C respectively. The second heating also revealed three melting peaks, at 45.1°C, at 53.7°C, and at 58.8°C (Figure 58). The appearance of the new peaks indicated the formation of vary crystalline forms. Crystalline forms have an impact on physical properties [114] and often polymorphic forms differ in melting points [115].

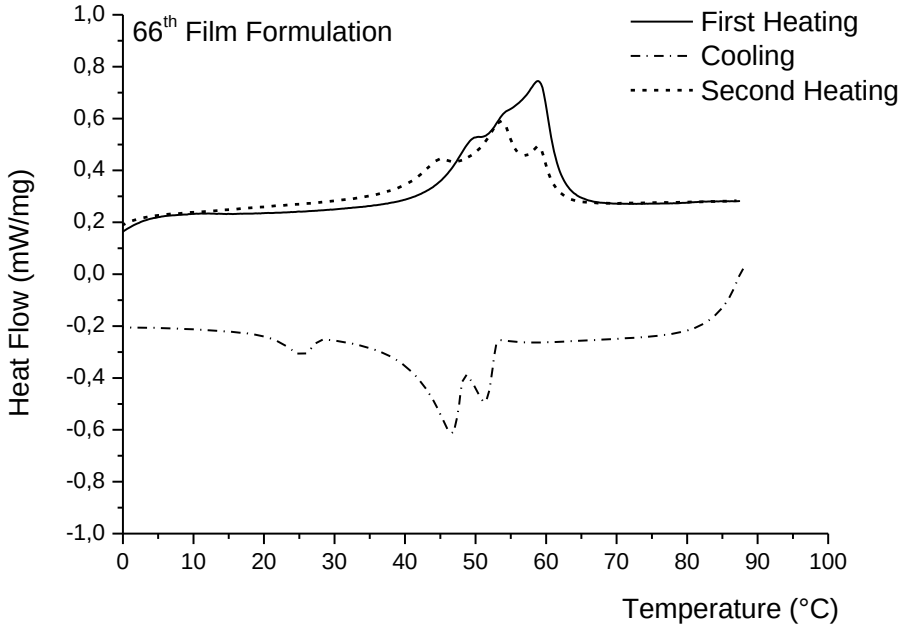


Figure 58. Differential Scanning Calorimetry thermogram of the 66th film formulation.

3.5.2. Differential Scanning Calorimetry analysis of 67th formulation

The DSC analysis of the 67th coating formulation is presented in Figure 59. After the first heating the whole investigated sample melted at 59.6°C. Besides this peak another heat effect at 51.5°C was detected. This could indicate the existence of polymorphic forms. During cooling one crystallization point at 49.4°C was observed. After the second heating one melting peak at 55.5°C was present.

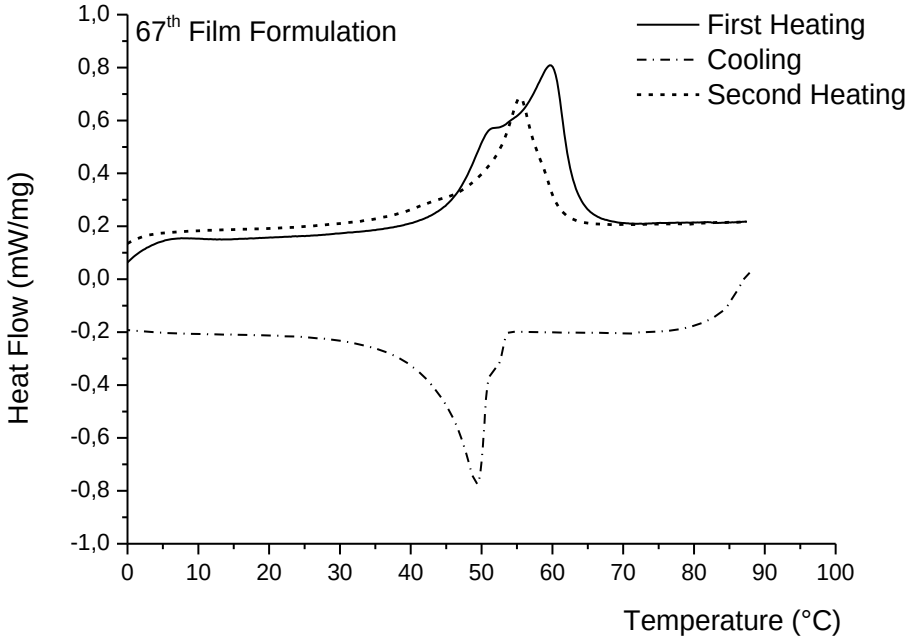


Figure 59. Differential Scanning Calorimetry thermogram of the 67th film formulation.

3.5.3. Differential Scanning Calorimetry analysis of 68th formulation

During the first heating a melting peak at 59.0°C appeared (Figure 60). During the cooling three solidification points at 50.0°C, at 44.7°C and at 25.2°C were detected, indicating the occurrence of different polymorphic forms. Also during the second heating three melting points at 46.7°C, at 52.5°C and at 59.3°C were revealed.

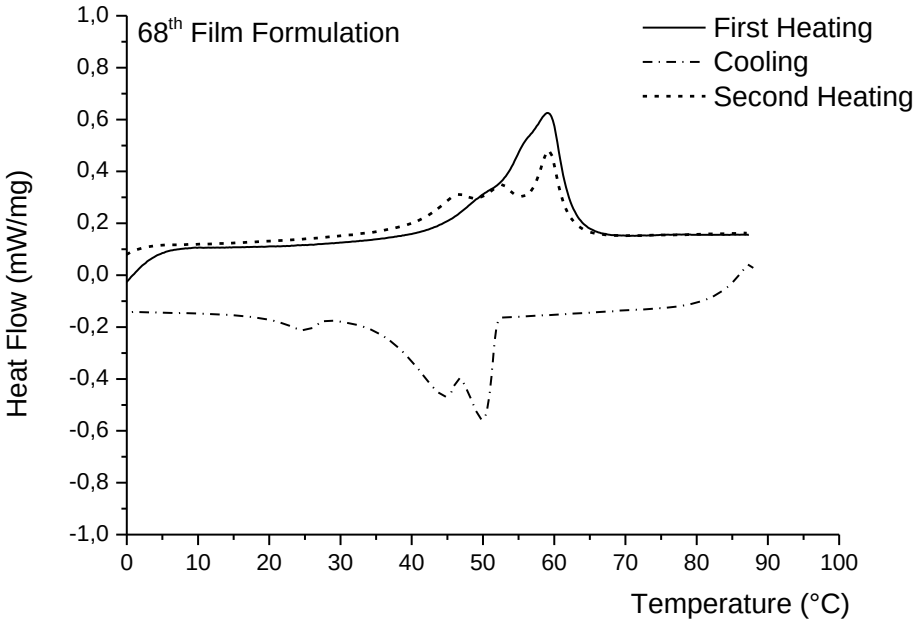


Figure 60. Differential Scanning Calorimetry thermogram of the 68th film formulation.

3.5.4. Differential Scanning Calorimetry analysis of 69th formulation

The investigated sample showed one melting peak at 58.2°C during the first heating (Figure 61). During crystallization two peaks were detected, at 49.6°C and at 44.1°C. During second heating of the sample three melting points at 46.0°C, at 51.8°C and at 58.2°C were observed. It could be assumed that temperature changes led to the formation of polymorphic forms.

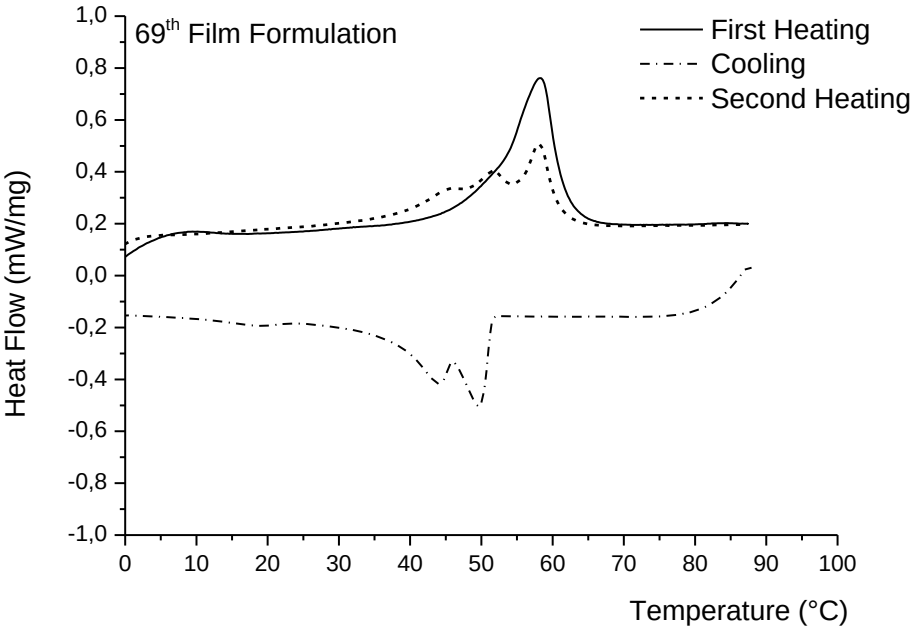


Figure 61. Differential Scanning Calorimetry thermogram of the 69th film formulation.

3.6. Dissolution tests of pellets coated with 66th-69th formulation after storage

Uncoated MCC-caffeine pellets and pellets coated with 66th, 67th, 68th and 69th formulation (coating thickness of 13.9 mg/cm^2) were stored at room temperature, protected from light over about 10.5 weeks. Subsequently, the dissolution tests of these pellets were performed. So the stability of the coating formulations was investigated.

The release of caffeine from uncoated pellets had the same rate both in hydrochloric acid (pH 1.2) medium and in phosphate buffer (pH 6.8). Thus, the release of the model drug took place in a pH-independent way. The release characteristic of uncoated pellets did not change after the storage period (Figure 62). In this manner the pellets were suitable to use for stability studies.

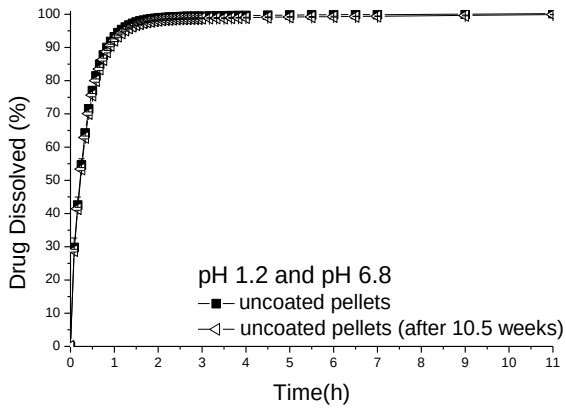


Figure 62. Release profile of caffeine from uncoated pellets right after production and after their storage for about 10.5 weeks in hydrochloric acid medium (pH 1.2) and phosphate buffer (pH 6.8).

The graphs of caffeine dissolution from the coated pellets with the developed lipid-based formulations after storage are presented below. For comparison on all graphs the release profiles of caffeine from the uncoated pellets and from the pellets analyzed directly after coating are also demonstrated. The release profile of caffeine from the uncoated pellets showed in the dissolution graphs is an average of all obtained release profiles of caffeine in HCl medium and in the phosphate buffer.

3.6.1. Dissolution tests of pellets coated with 66th formulation

Over the first hour of the dissolution test the amount of caffeine released from the stored pellets (F66$t_{10.5w}$) in hydrochloric acid medium (pH 1.2) was 2.1 %. After 2 hours' incubation the quantity of the active substance increased to 7.0 %. Over the following hour 26.7 % of caffeine were released (Figure 63).

After the first hour no significant differences in the quantity of released caffeine between the pellets analyzed after the coating (F66t_0, Figure 50) and the pellets analyzed after the storage (F66$t_{10.5w}$) in HCl medium could be observed. After the next half an hour the difference increased only slightly. The released quantity of caffeine was 3.0 % for F66t_0 and 3.6 % for F66$t_{10.5w}$.

After 2 hours the differences between the release profiles grown to ca. 2.5 %. Then the differences were increasing significantly.

The release profile of caffeine from the pellets coated with 66th formulation after 10.5 weeks storage (F66t$_{10.5w}$) in phosphate buffer (pH 6.8) is shown in Figure 64. After 60 minutes the quantity of released caffeine was 36.8 %. In the second hour 85.2 % of the model drug were already detected. After 3 hours the released amount of API reached the value of 95.7 %.

The release profiles of caffeine from lipid coated pellets analyzed after coating (F66t$_0$, Figure 51) and after 10.5 weeks storage (F66t$_{10.5w}$) in the phosphate buffer (pH 6.8) were very similar.

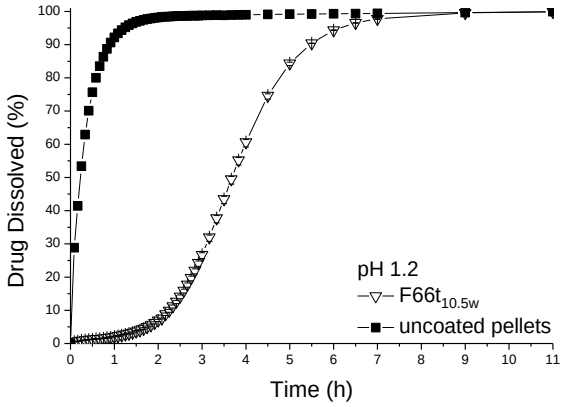


Figure 63. Release profile of caffeine from uncoated pellets, and from pellets coated with 66th formulation after 10.5 weeks' storage (F66t$_{10.5w}$) in hydrochloric acid medium (pH 1.2).

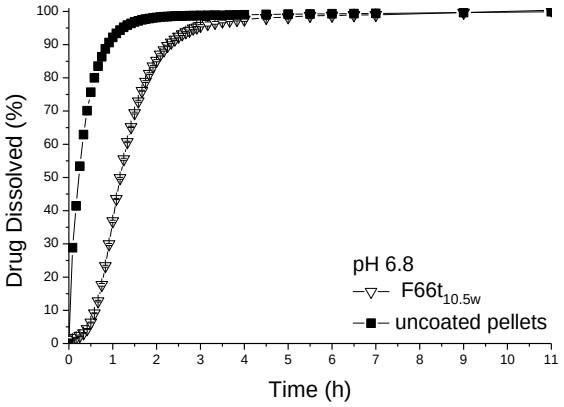


Figure 64. Release profile of caffeine from uncoated pellets and from pellets coated with 66th formulation after 10.5 weeks' storage (F66t$_{10.5w}$) in phosphate buffer (pH 6.8).

3.6.2. Dissolution tests of pellets coated with 67th formulation

The amount of caffeine released in hydrochloric acid medium (pH 1.2) from the stored pellets (F67t$_{10.5w}$) after one hour incubation was 2.4 %. Over the following hour the amount increased up to 21.2 %. Over 3 hours already 71.4 % of the active substance were detected (Figure 65).

Only during the first hour of the dissolution test the release profiles of the coated pellets analyzed right after their coating (F67t$_0$, Figure 56) and of the pellets analyzed after their storage (F67t$_{10.5w}$) in HCl medium were quite similar. Since then the differences were rising. After 2 hours about 8.8 % of caffeine more were released from the F67t$_{10.5w}$ than from F67t$_0$.

In phosphate buffer the quantity of released model drug from the coated pellets stored for 10.5 weeks after one hour was 45.3 %. After 2 hours already 87.9 % were detected. One hour later 95.2 % of API were released (Figure 66).

The release rate of caffeine in phosphate buffer after pellets storage for 10.5 weeks (F67t$_{10.5w}$) practically did not change compared to the release rate of caffeine from pellets analyzed directly after their coating (F67t$_0$, Figure 57). Both the release profiles matched almost exactly.

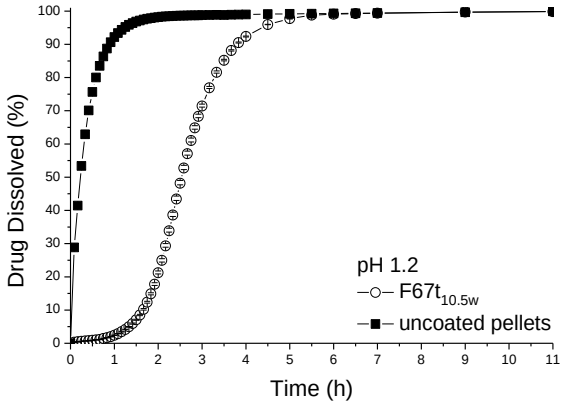


Figure 65. Release profile of caffeine from uncoated pellets and from pellets coated with 67th formulation after 10.5 weeks' storage ($F67t_{10.5w}$) in hydrochloric acid medium (pH 1.2).

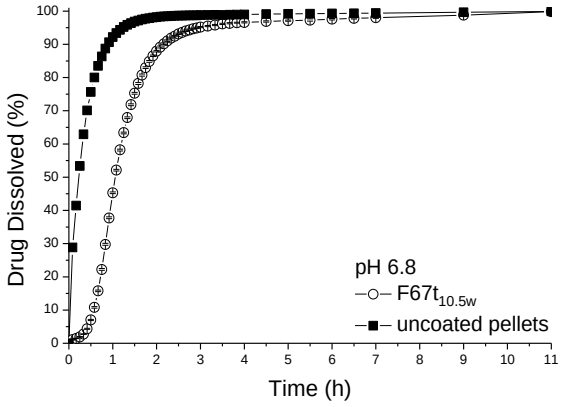


Figure 66. Release profile of caffeine from uncoated pellets and from pellets coated with 67th formulation after 10.5 weeks' storage ($F67t_{10.5w}$) in phosphate buffer (pH 6.8).

3.6.3. Dissolution tests of pellets coated with 68th formulation

Over the first hour of the stored pellets' incubation ($F68t_0$) in hydrochloric acid medium (pH 1.2) 1.8 % of caffeine were released. After 2 hours the amount of the released drug had a value of 9.0 %. Over the following hour the observed quantity of caffeine was 29.8 % (Figure 67).

During the first hour of measurements in HCl medium the active substance was released from the stored pellets ($F68t_{10.5w}$) and from the pellets analyzed directly after coating ($F68t_0$, Figure 58) in a similar way. Then the differences were steadily growing. After 2 hours 2.6 % more caffeine were released from $F68t_{10.5w}$.

After the first hour of the dissolution test in phosphate buffer 35.6 % of caffeine from the coated pellets after storage were released. Over the following hour 81.4 % of API were already detected. After 3 hours the amount of the released model drug was 93.1 % (Figure 68).

The dissolution rates in phosphate buffer of caffeine from the pellets analyzed directly after coating ($F68t_0$, Figure 59) and after storage for 10.5 weeks ($F68t_{10.5w}$) were quite similar. The dissolution curves of the active substance varied only minimally.

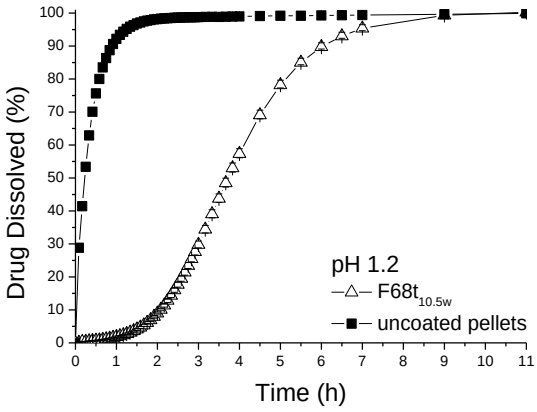


Figure 67. Release profile of caffeine from uncoated pellets and from pellets coated with 68th formulation after 10.5 weeks' storage ($F68t_{10.5w}$) in hydrochloric acid medium (pH 1.2).

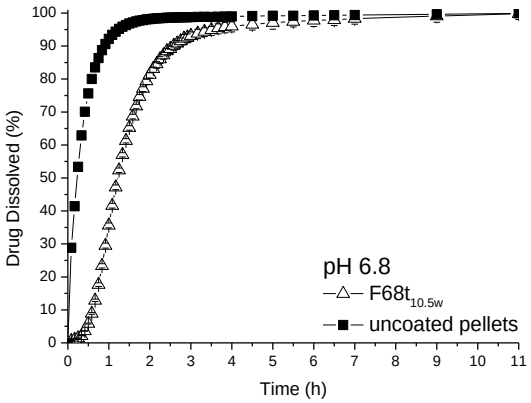


Figure 68. Release profile of caffeine from uncoated pellets and from pellets coated with 68th formulation after 10.5 weeks' storage ($F68t_{10.5w}$) in phosphate buffer (pH 6.8).

3.6.4. Dissolution tests of pellets coated with 69th formulation

After one hour of the dissolution test of stored pellets ($F69t_{10.5w}$) in hydrochloric acid medium (pH 1.2) only 1.0 % of the model drug was released. Over following hour the detected amount of caffeine was 4.2 %. After 3 hours 13.3 % of caffeine could be noted (Figure 69).

Till the first two hours the course of the dissolution profiles of the active substance from the pellets analyzed right after their coating ($F69t_0$, Figure 60) and from stored pellets ($F69t_{10.5w}$) in HCl medium was very similar. Afterwards the dissolution rate of $F69t_{10.5w}$ was growing faster than the dissolution rate of $F69t_0$.

The quantity of released API from the coated pellets after storage in the phosphate buffer was 29.1 %. After 2 hours 73.5 % were already released. Over the following hour 88.0 % of caffeine were detected (Figure 70).

The release rates of the model drug of the pellets analyzed directly after coating ($F69t_0$, Figure 61) and after storage ($F69t_{10.5w}$) in the phosphate buffer differed only slightly.

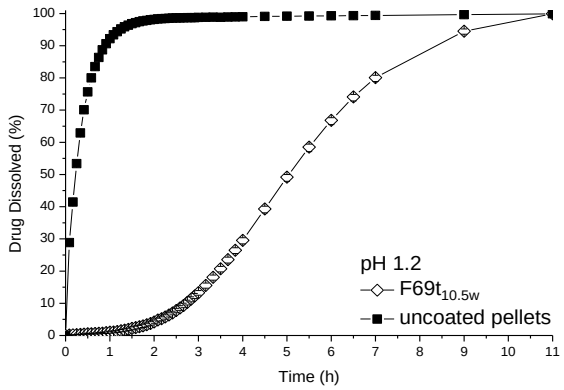


Figure 69. Release profile of caffeine from uncoated pellets and from pellets coated with 69th formulation after 10.5 weeks' storage ($F69t_{10.5w}$) in hydrochloric acid medium (pH 1.2).

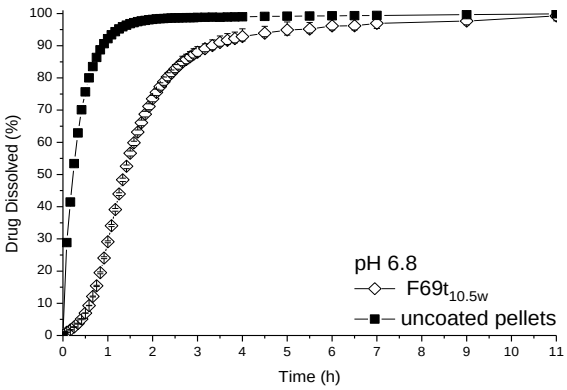


Figure 70. Release profile of caffeine from uncoated pellets and from pellets coated with 69th formulation after 10.5 weeks' storage ($F69t_{10.5w}$) in phosphate buffer (pH 6.8).

3.6.5. Screening of coating formulations stability-summary

After the storage period (10.5 weeks, at room temperature, light-protected) the release rate of caffeine from the pellets coated with developed lipid formulations ($F66t_{10.5w}$-$F69t_{10.5w}$) changed both in hydrochloric acid medium (pH 1.2) and in phosphate buffer (6.8). The release rates of stored pellets ($F66t_{10.5w}$-$F69t_{10.5w}$, figures 63-70) were higher than the release rates obtained from the pellets analyzed right after their coating ($F66t_0$-$F69t_0$, figures 50-57). However, during 2 hours' incubation in HCl medium the release profiles of $F66t_{10.5w}$-$F69t_{10.5w}$ did not differ much compared to the release profiles of $F66t_0$-$F69t_0$. Thereafter, the differences were growing. Nonetheless three out of four developed coatings could still be potentially used for the release delay of active substances in gastro-resistant dosage forms. The dissolution graphs in the phosphate buffer of the pellets analyzed directly after coating ($F66t_0$-$F69t_0$) and of the pellets analyzed after their storage ($F66t_{10.5w}$-$F69t_{10.5w}$) differed only slightly from each other for the whole test period.

The shortest lag phase in hydrochloric acid medium was shown by the pellets coated with the 67th formulation ($F67t_{10.5w}$). The longest, in turn, was observed for the pellets coated with the 69th formulation ($F69t_{10.5w}$). Pellets coated with the two other lipid formulations ($F66t_{10.5w}$ and $F68t_{10.5w}$) demonstrated quite similar release characteristic.

The differences in the release rates of the stored pellets coated with lipid coating formulations ($F66t_{10.5w}$-$F69t_{10.5w}$) in phosphate buffer (pH 6.8) were very small. Compared to the uncoated pellets the release of caffeine from the coated pellets was delayed. However, the release delays were considerably smaller compared to the release delays in HCl medium.

After the first hour of the dissolution test in HCl medium the differences in the amount of released caffeine between the stored pellets and the pellets analyzed directly after coating ranged from 0.3 %, for the 66th formulation, to 0.8 %, for the 67th formulation. After following hour the smallest difference exhibited the 69th formulation, namely 1.9 %. For this formulation the released amount of the model drug had also the lowest value of 4.2 %. In the meantime the 67th formulation released instead 21.2 % showing thereby the highest release rate. After 3 hours the released quantity of caffeine from the 69th formulation was 13.3 % whereas the 67th formulation released already 71.4 %. The remaining two formulations (the 66th and the 68th formulations) released almost 30 % of caffeine.

The release rates of the model drug from the stored pellets coated with developed lipid formulations (F66$t_{10.5w}$-F68$t_{10.5w)}$ in phosphate buffer were much higher than the release rates in hydrochloric acid medium. During the first hour of the dissolution test the quantity of released caffeine from the stored pellets ranged from 29.1 % for the 69th formulation, to 45.3 % for the 67th formulation. After 2 hours' incubation the formulations F66$t_{10.5w}$-F68$t_{10.5w}$ released higher than 80 % of the active substance. Only the F69$t_{10.5w}$ formulation released less than 80 % of caffeine. Over the following hour the three first formulations released more than 90 % of caffeine. The last formulation released 88.0 % of the model drug.

3.7. Development of lipid coating formulations- summary and conclusions

The development process of the coating formulations based on lipids was very complex. Alone the selection of adequate components was extremely time-consuming. On the one hand, the excipients must have been able to create a sprayable coating dispersion; on the other hand the film formed on the pellets surface had to modify the release of the active substance in a desirable way.

As a result of the present work four coating formulations based on lipids were developed. The ingredients of these formulations were able to create suitable coating dispersions being capable of forming a coherent film on pellet surface. The dissolution studies demonstrated that these coatings ensured a modified release of the model drug. The drug release occurred in a pH- and time-dependent way. In hydrochloric acid medium (pH 1.2) all pellets coated with developed lipid formulations released the drug after a defined lag phase. Therefore, the invented coatings can be used for development of delayed-release dosage forms. Moreover, the 66th, 68th and 69th formulations can potentially act as gastro-resistant coatings. In phosphate buffer (pH 6.8) the release profiles of the active substance from lipid coated pellets significantly differed from these

in HCl medium. Here, the release rates of API for all formulations were much higher. However, the dissolution of caffeine was still slower than its dissolution from uncoated pellets.

It could be supposed that the API release under in vivo conditions will differ from the investigated API release under in vitro conditions. The release rates of lipid coated pellets in the stomach could be somewhat higher than the release rates in the hydrochloric acid medium. On the other hand, in the small intestine a much faster drug release than in phosphate buffer can be expected. The used dissolution media during in vitro dissolution tests (HCl medium and phosphate buffer) did not contain any digestive enzymes. In contrast, in human gastro-intestinal tract digestive enzymes are present, including lipases responsible for the digestion of lipids. There are two main enzymes involved in the digestion of lipids, namely gastric lipase, present in the gastric juice, and pancreatic lipase, secreted into the duodenum [116]. The main lipid digestion occurs in the small intestine. The level of the lipid digestion in stomach is much lower than in the small intestine. Human gastric lipase shows less efficiency than human pancreatic lipase, because of the lower amounts of enzyme present [116]. The total activity of the gastric lipase amounts to ca. 20% of the pancreatic lipase [117]. Monoglycerides and free fatty acids, which are a part of the developed coatings, could also interact with the bile salts and phospholipids present in the small intestine forming mixed micelles. So, the API release could be accelerated. In this manner the differences in the release rates between in vivo and in vitro conditions could be explained.

Screenings of coating formulations' stability revealed changes in the release profiles of the active substance after the storage of pellets for 10.5 weeks (at room temperature, light-protected). The release rates of caffeine from all the formulations stored ($F66t_{10.5w}$-$F69t_{10.5w}$) increased. However, the pH-dependent and delayed release characteristic could still be observed. Moreover, the differences in the release profiles between pellets analyzed directly after coating ($F66t_0$-$F69t_0$) and pellets analyzed after their storage ($F66t_{10.5w}$-$F69t_{10.5w}$) during the first 2 hours of the dissolution test in HCl medium were insignificant. Not until then the release rates were steadily growing.

The developed lipid-based coatings could be an alternative for shellac coatings used in enteric drug delivery systems. Shellac shows good resistance in gastric fluids, but a considerable problem is the slow dissolution of shellac coatings in higher pH media, such as intestinal fluids [22]. The developed lipid coatings provide a good barrier in gastric environment and their permeability significantly increase in higher pH value (intestinal milieu). Additionally, each of the coating ingredients is commonly consumed with everyday foods. Thus, the developed formulations will be willingly applied in nutraceuticals.

3.8. Characterization of 69th lipid-based coating formulation

Pellets coated with 69th coating dispersion exhibited the longest lag-phase among all the formulations created. Furthermore, these pellets were characterized by the highest stability. The difference of the released caffeine between pellet analyzed directly after coating ($F69t_0$) and pellets stored for 10.5 weeks at room temperature ($F69t_{10.5w}$) after 2 hours of the dissolution test in HCl medium was the smallest compared to the other formulations. For these reasons, the 69th coating formulation was chosen for more detailed analyses.

For the screening purposes pellets prepared via saturation technique were absolutely sufficient to give the first view about the coating formulation's properties. However, for more detailed characteristics of the coating, the preparation of pellets with the defined content of an active substance was necessary. These pellets were produced using extrusion/spheronization method. For further examinations, the pellets within the class range 1000-1400 µm were used. In order to separate other pellet fractions the granules were sieved. After sieving the pellets were coated with the 69th formulation (coating thickness of 13.9 mg/cm^2). The performance of the coating process was the same as in the case of the pellets prepared by the saturation technique (see Chapter 2.2.5). Subsequently, a part of the coated pellets was directly subjected to dissolution tests ($F69't_0$). The other parts of the coated pellets were subjected to dissolution tests after various periods of storage under different humidity and temperature conditions. In this way, an impact of storage conditions and storage period on the model drug release from pellets coated with the 69th formulation could be evaluated.

3.8.1. Pellets manufactured using extrusion/spheronization method

Pellets containing caffeine as a model drug were manufactured using extrusion/spheronization method. The aim was to obtain particles with a narrow size distribution, preferably close to the size of cellets 1000® used for the screening purposes, falling into the class range 1000-1400 µm. At the same time, the produced pellets must release the active substance immediately after their placement in a dissolution medium in a pH-independent manner. Fast drug release from produced spheres was assured by an adequate pellets composition. One possibility to obtain fast drug release from MCC-based pellets is the incorporation of water soluble fillers [118]. Therefore, besides the active substance and microcrystalline cellulose, lactose monohydrate as water soluble filler was used. Fast and pH-independent caffeine release from manufactured pellets allowed the observation of possible modification of the API release through the lipid-based film and a reliable evaluation of the developed coating formulation.

3.8.2. Sieve analysis

The sieve analysis was performed for caffeine pellets prepared using extrusion/spheronization method. The manufactured pellets had a very narrow particle size distribution (Figure 71). Two main fractions of pellets could be distinguished. About 43 % of the granules were in the class range 1000-1400 µm. The other main fraction of pellets, ca. 42 %, had a particle size distribution of 1400-2000 µm. In the class range 710-1000 µm about 11 % of granules were found. About 5 % of pellets were in the last size class 2000-2800 µm. This class consisted only of pellet agglomerates. For coating and dissolution test purposes pellets with particle size distribution of 1000-1400 µm were used.

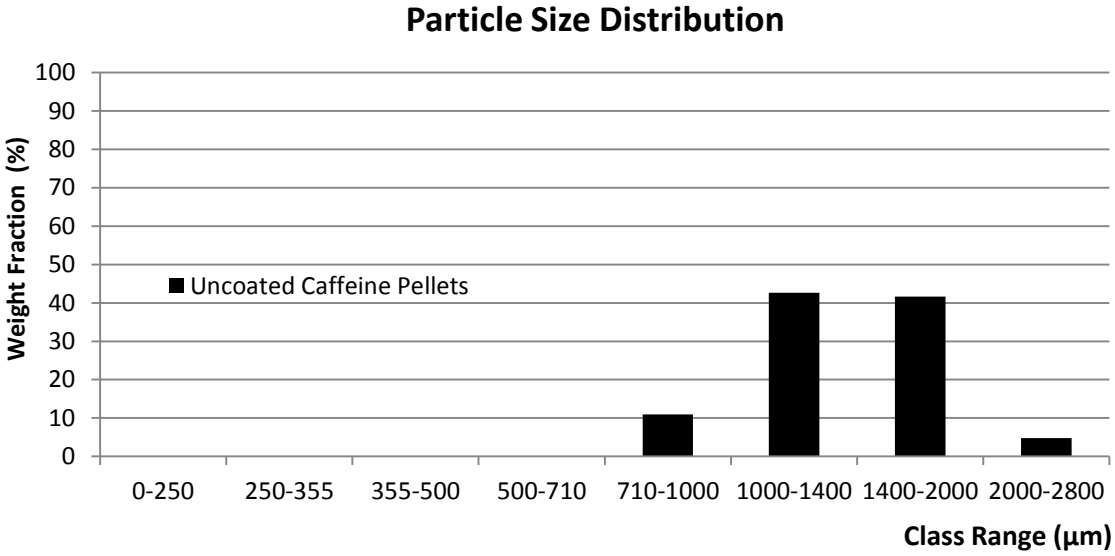


Figure 71. Particle size distribution of caffeine pellets prepared via extrusion/spheronization method.

3.8.3. Dissolution tests of uncoated caffeine pellets

The dissolution tests of uncoated pellets produced via extrusion/spheronization method were performed in hydrochloric acid medium (pH 1.2), in phosphate buffer (pH 6.8) and in distilled water. The dissolution profiles are presented in Figure 72. The release of the active substance after 30 minutes was almost complete in each medium. Moreover, the release of caffeine was not dependent on pH value. Therefore these pellets are suitable for the further evaluation of the coating formulation.

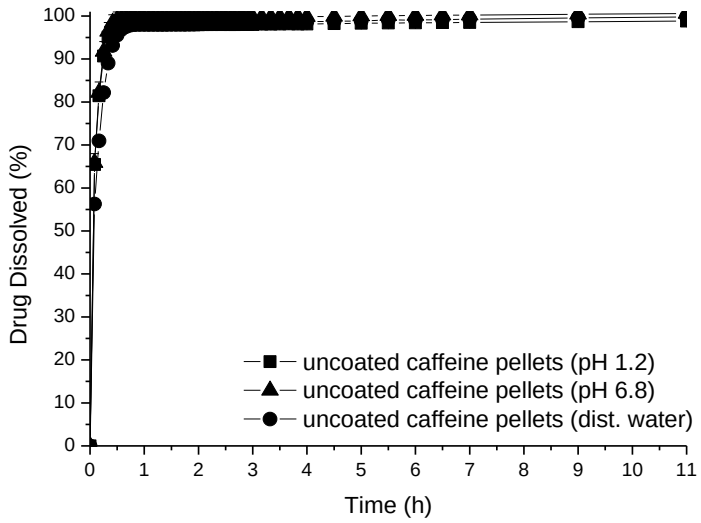


Figure 72. Dissolution kinetic of caffeine released from uncoated pellets in HCl medium (pH 1.2), in phosphate buffer (pH 6.8) and in distilled water.

3.8.4. Dissolution tests of coated pellets (directly after coating)

Pellets directly after coating with the 69th formulation (F69't_0, coating thickness of 13.9 mg/cm^2) were subjected to dissolution tests in two dissolution media. The quantity of released caffeine from lipid coated pellets in hydrochloric acid medium (pH 1.2) after 1 hour amounted to 0.4 % only. After the following hour the quantity increased to 1.5 %. After 3 hours of the dissolution test 4.3 % of caffeine were released (Figure 73).

In phosphate buffer (pH 6.8) 20.4 % of the drug were released over the first hour. After the following hour the quantity of released caffeine amounted 73.9 %. After 3 hours already 93.3 % of the drug were released (Figure 74).

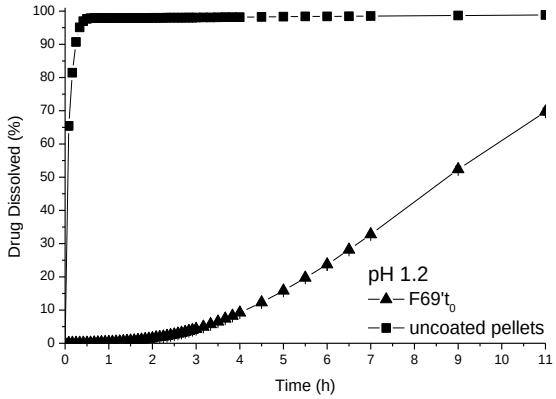


Figure 73. Dissolution kinetic of caffeine released from uncoated and lipid coated pellets analyzed directly after coating (F69't_0) in HCl medium (pH 1.2). Pellets were produced using extrusion/spheronization method.

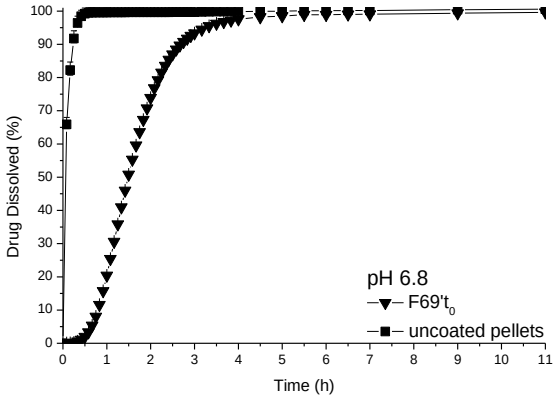


Figure 74. Dissolution kinetic of caffeine released from uncoated and lipid coated pellets analyzed directly after coating (F69't_0) in phosphate buffer (pH 6.8). Pellets were produced using extrusion/spheronization method.

3.8.5. Dissolution tests after pellets storage under different conditions

Pellets coated with 69th formulation were stored under different temperature and humidity conditions. Subsequently, after various periods of storage pellets were subjected to dissolution tests.

Storage at 33 % RH for two weeks (21°C)

After the first hour of the test in HCl medium 0.7 % of caffeine from lipid coated pellets (F69'$t_{2w33\%RH}$) were released. Within the following hour the amount of released API had a value of 2.5 %. During 3 hours of pellets incubation the value of released caffeine increased to 6.97 % (Figure 75).

The amount of released caffeine in phosphate buffer after one hour was 25.8 %. After 2 hours 80.2 % were already released. One hour later the detected quantity of released API had a value of 95.7 % (Figure 76).

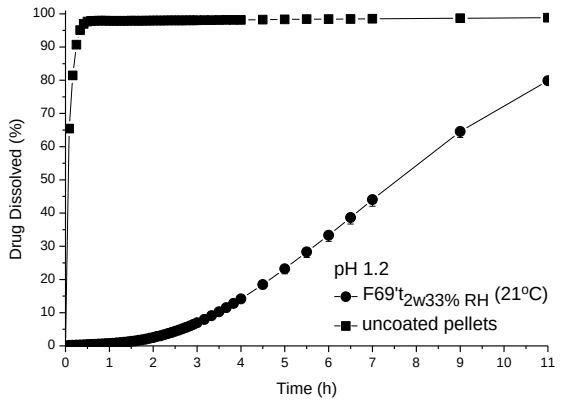


Figure 75. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 2 weeks storage at 33 % RH, 21°C ($F69't_{2w33\%RH}$) in hydrochloric acid medium (pH 1.2).

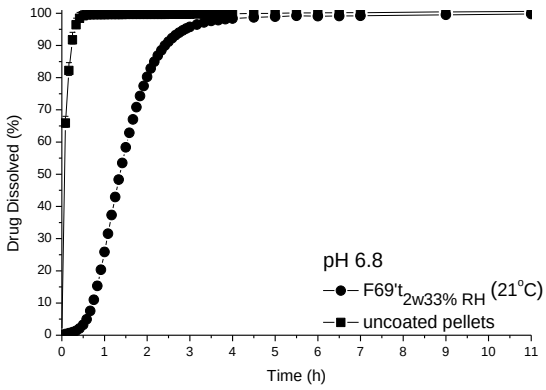


Figure 76. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 2 weeks storage at 33 % RH, 21°C ($F69't_{2w33\%RH}$) in phosphate buffer (pH 6.8).

Storage at 76 % RH for two weeks (21°C)

In hydrochloric acid medium (pH 1.2) during the first hour 1.3 % of the active substance from stored pellets ($F69't_{2w76\%RH}$) were released. After 2 hours of the dissolution test 4.0 % of caffeine were detected. The measured amount of caffeine after following hour was 9.5 % (Figure 77).

The amount of released caffeine from coated pellets in phosphate buffer after 1 hour incubation was 29.7 %. Within the following hour the amount grew to 82.5 %. Already 96.1 % of the API were released after 3 hours (Figure 78).

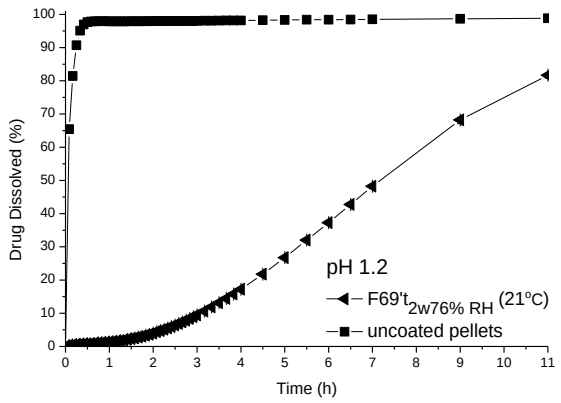


Figure 77. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 2 weeks storage at 76 % RH, 21°C ($F69't_{2w76\%RH}$) in hydrochloric acid medium (pH 1.2).

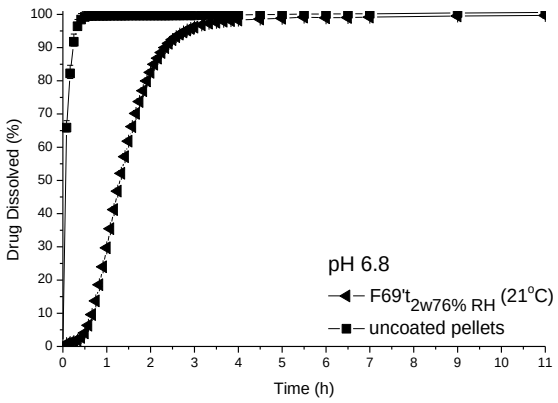


Figure 78. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 2 weeks storage at 76 % RH, 21°C ($F69't_{2w76\%RH}$) in phosphate buffer (pH 6.8).

Storage for two weeks at different humidity conditions-comparison

After two weeks' period both pellets stored at 33 % RH (F69't$_{2w33\%RH}$) and at 76 % RH (F69't$_{2w76\%RH}$) released the model drug in HCl medium faster than pellets analyzed directly after coating (F69't$_0$, Figure 73). However, pellets stored at lower humidity (F69't$_{2w33\%RH}$) showed slower release rate than pellets stored at higher humidity (F69't$_{2w76\%RH}$). Over the first 2 hours the release rate of the active substance from F69't$_0$ and from F69't$_{2w33\%RH}$ in HCl medium differed only slightly. Subsequently, the differences were constantly growing. The release rate of F69't$_{2w76\%RH}$ started to differ from the release rate of F69't$_0$ already after the first hour of the test.

In phosphate buffer the release rate of API from pellets analyzed after their coating (F69't$_0$, Figure 74) was lower than from pellets stored both at 33 % RH (F69't$_{2w33\%RH}$) and at 76 % RH (F69't$_{2w76\%RH}$). However, the differences between the dissolution profiles were very small. After 3 hours the released amount of caffeine was more than 90 % for each formulation. The release profiles of caffeine from F69't$_{2w33\%RH}$ and from F69't$_{2w76\%RH}$ showed practically the same course.

The results indicate the influence of storage conditions on the drug release characteristics. This fact should be taken into consideration during further formulation elaboration and while choosing storage conditions of coated dosage forms with the lipid formulation. Nonetheless, after the storage, the delayed and pH-dependent release characteristic of the model drug still remained.

Storage at 33 % RH for six weeks (21°C)

After the first hour of the dissolution test 0.7 % of caffeine from stored pellets (F69't$_{6w33\%RH}$) in hydrochloric acid medium (pH 1.2) was released. The amount of detected API after 2 hours was 2.1 %. Over the following hour the amount grew to 5.9 % (Figure 79).

After the storage the quantity of released caffeine in phosphate buffer amounted to 24.2 %. Within the following hour 80.5 % of the model drug were already detected. Over 3 hours 96.2 % of caffeine were released (Figure 80).

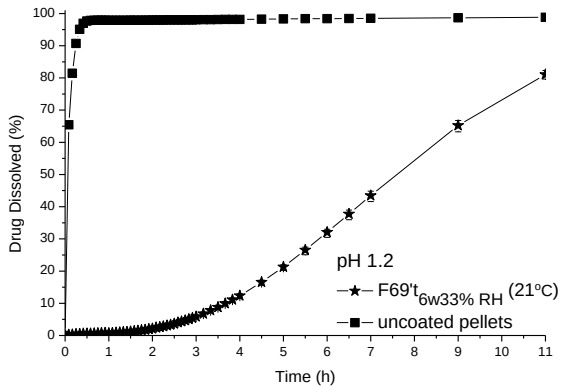


Figure 79. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 6 weeks storage at 33 % RH, 21°C ($F69't_{6w33\%RH}$) in hydrochloric acid medium (pH 1.2).

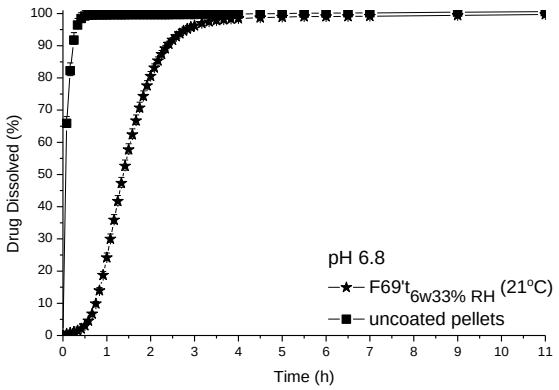


Figure 80. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 6 weeks storage at 33 % RH, 21°C ($F69't_{6w33\%RH}$) in phosphate buffer (pH 6.8).

Storage at 76 % RH for six weeks (21°C)

After the first hour of stored pellets incubation ($F69't_{6w76\%RH}$) 1.8 % of caffeine in HCl medium were detected. In the next hour the amount increased to 3.7 %. After 3 hours 7.7 % of the model drug were released (Figure 81).

In phosphate buffer the detected amount of caffeine after 1 hour of the dissolution test was 26.2 %. After 2 hours the quantity of released caffeine amounted to 81.1 %. After the following hour 96.5 % of the active substance were already released (Figure 82).

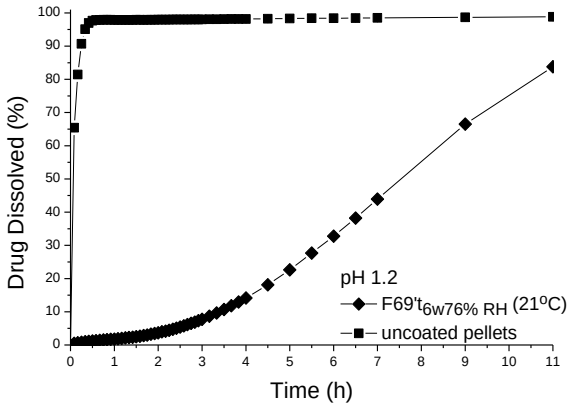


Figure 81. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 6 weeks storage at 76 % RH, 21°C ($F69't_{6w76\%RH}$) in hydrochloric acid medium (pH 1.2).

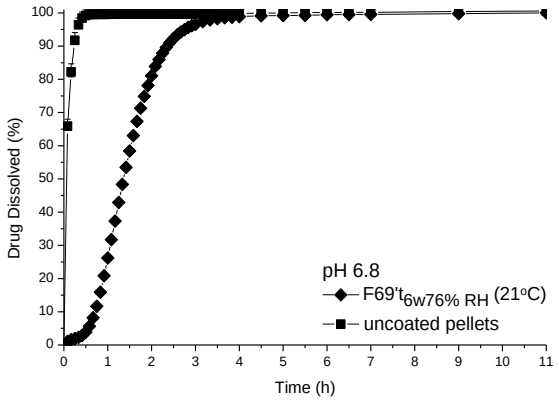


Figure 82. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 6 weeks storage at 76 % RH, 21°C ($F69't_{6w76\%RH}$) in phosphate buffer (pH 6.8).

Storage at 10°C for six weeks (in a fridge, no humidity-controlled storage)

Only 0.6 % of caffeine was released in HCl medium (pH 1.2) from pellets stored for six weeks at 10°C (in the fridge, F69't$_{6w10°C}$). After 2 hours of the test 1.7 % of the model drug were detected. Over 3 hours the quantity of caffeine amounted to 4.4 % (Figure 83).

After 1 hour the amount of released caffeine in phosphate buffer (pH 6.8) from coated pellets stored for six weeks at 10°C (in the fridge, F69't$_{6w10°C}$) was 21.9 %. Within the next hour the amount increased to 77.6 %. During 3 hours of the dissolution test 95.1 % of the model drug were released (Figure 84).

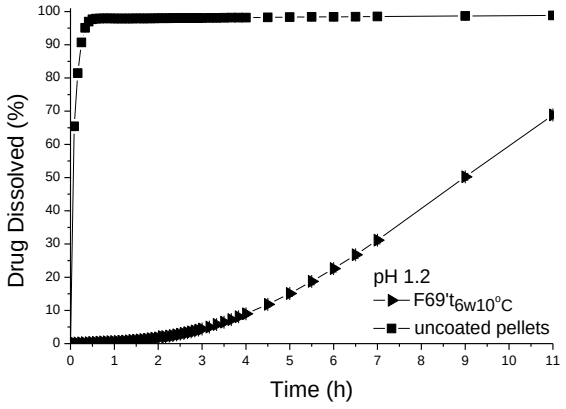


Figure 83. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 6 weeks storage at 10°C (fridge, 69't$_{6w10°C}$) in hydrochloric acid medium (pH 1.2).

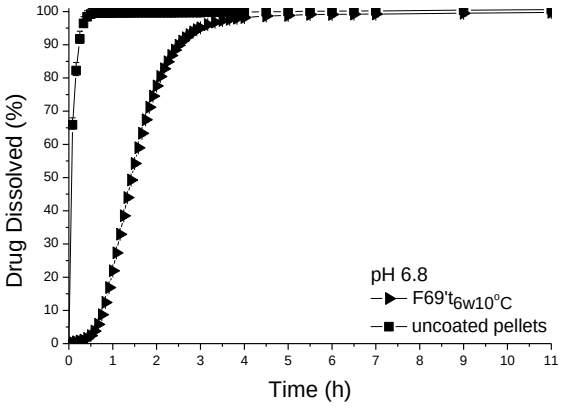


Figure 84. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th formulation after 6 weeks storage at 10°C (fridge, 69't$_{6w10°C}$) in phosphate buffer (pH 6.8).

Storage at 33 % RH for different time period-comparison

Over the first 2 hours the release rate in acidic medium (pH 1.2) from pellets stored at 33 % RH (F69't$_{2w33\%RH}$ and F69't$_{6w33\%RH}$) and from pellets analyzed right after coating (F69't$_0$, Figure 73) was nearly identical. Afterwards, the changes were increasing. Formulation F69't$_{2w33\%RH}$ and F69't$_{6w33\%RH}$ released the active substance practically in the same meaner. The differences in the release rate were insignificant.

In phosphate buffer (pH 6.8) the release of caffeine from pellets stored for two and for six weeks at 33 % RH (F69't$_{2w33\%RH}$ and F69't$_{6w33\%RH}$) had approximately the same pattern. Pellets analyzed directly after coating (F69't$_0$, Figure 74) released the model drug only slightly more slowly.

Storage at 76 % RH for different time period-comparison

Pellets stored at 76 % RH both for two (F69't$_{2w76\%RH}$) and for six weeks (F69't$_{6w76\%RH}$) released the active substance in HCl medium (pH 1.2) faster than pellets analyzed directly after coating (F69't$_0$, Figure 73). The fastest release of caffeine was found in pellets stored for six weeks at 76 % RH (F69't$_{6w76\%RH}$). However, over the first 2 hours no significant differences between the release profiles of formulation F69't$_{2w76\%RH}$ and F69't$_{6w76\%RH}$ were visible. Since then the distinctions were somewhat growing.

The dissolution profiles of caffeine released from pellets stored for two (F69't$_{2w76\%RH}$) and for six weeks (F69't$_{6w76\%RH}$) at 76 % RH in phosphate buffer (pH 6.8) were highly comparable. Moreover, formulation F69't$_0$ (Figure 74) demonstrated only slightly slower release compared to formulations F69't$_{2w76\%RH}$ and F69't$_{6w76\%RH}$.

Storage for six weeks at different humidity and temperature conditions-comparison

After six weeks' storage of coated pellets at 33 % RH (F69't$_{6w33\%RH}$) and at 76 % RH (F69't$_{6w76\%RH}$) the release rates of caffeine in hydrochloric acid medium (pH 1.2) increased compared to the release rate of pellets analyzed directly after coating (F69't$_0$, Figure 73). However, within 2 hours the release profile of pellets stored at lower humidity (F69't$_{6w33\%RH}$) was very similar to release profile of F69't$_0$. Subsequently, the differences were gradually more significant. For pellets stored at higher humidity (F69't$_{6w76\%RH}$) the release rate was the highest. Nonetheless, the differences in the dissolution profiles between F69't$_{6w33\%RH}$ and F69't$_{6w76\%RH}$ were very slight. The release profile of pellets stored in the fridge (10°C, F69't$_{6w10°C}$) was practically like the release profile of F69't$_0$. Up to 4 hours the amount of released caffeine practically did not differ. Since then the differences were after all insignificant.

In phosphate buffer (pH 6.8) the release profiles of stored pellets at different humidity and temperature conditions (F69't$_{6w33\%RH}$, F69't$_{6w76\%RH,}$ F69't$_{6w10°C}$) and of pellets analyzed directly after coating (F69't$_0$, Figure 74) were very similar. The release rate of F69't$_0$ was only slightly lower than release rates of all stored pellets. Practically no differences were visible between release rates of F69't$_{6w33\%RH}$ and F69't$_{6w76\%RH}$. Pellets stored in the fridge (F69't$_{6w10°C}$) showed a little bit slower release compared to the pellets stored under controlled humidity conditions.

After analyzing the results presented above the impact of storage conditions on the release of the model drug from pellets coated with the 69th formulation could be observed. The release rate of caffeine in HCl medium (pH 1.2) increased after pellets storage at 33 % RH and 76 % RH. In contrast, pellets stored for six weeks in the fridge (10°C) released the active substance similarly to pellets analyzed directly after coating. The differences in caffeine release from all formulations in phosphate buffer (pH 6.8) were very small. The obtained results indicated that pellets coated with developed coating formulation should be stored at decreased temperature (e.g. in a fridge) in order to prevent changes in the release rate.

3.8.6. Dissolution tests after pellets storage under different conditions-summary

Having analyzed the dissolution kinetics presented above the influence of storage conditions on the release rate of the model drug from pellets coated with the 69th lipid formulation was revealed.

Analyses in hydrochloric acid medium (pH 1.2)

The greatest change in the release rate in comparison to the release rate of pellets analyzed directly after their coating (F69't_0) were observed for pellets stored at 76 % RH. However, after pellets storage at this humidity value the release of caffeine was only somewhat faster. Furthermore, comparing the release rate after two (F69'$t_{2w76\%RH}$) and six weeks (F69'$t_{6w76\%RH}$) no significant differences up to 2 hours were noted. Since then only small variations between the two profiles could be observed.

Pellets stored for two (F69'$t_{2w33\%RH}$) and for six weeks (F69'$t_{6w33\%RH}$) at 33 % RH showed practically the same release rate. Moreover, up to 2 hours the release profiles differed from the release profile of pellets analyzed after coating (F69't_0) insignificantly. Since gastro-resistant dosage forms have to demonstrate resistance to stomach acid during 2 hours, changes in release rate after 2 hours could be ignored.

After six weeks' pellets storage in the fridge (at 10°C, F69'$t_{6w10°C}$) basically no differences comparing the dissolution profile of pellets analyzed after coating (F69't_0) could be detected. Also after 2 hours the profiles showed very similar course. Therefore, the most recommended storage conditions for pellets coated with the 69th film formulation seem to be at decreased temperature, e.g. in the fridge. In conclusion, at lower storage temperature pellets coated with the developed lipid formulation showed satisfactory stability.

Analyses in phosphate buffer (pH 6.8)

After pellets storage at different temperature and humidity conditions the release profiles differed only slightly from the release profile of pellets analyzed right after coating (F69't_0). The highest release rate demonstrated pellets stored at 76 % RH. Only a little slower release was shown by pellets stored at 33 % RH. Pellets stored for two and for six weeks both at 33 % RH and at 76 % RH had practically the same release rate. Pellets stored in the fridge (at 10°C, F69'$t_{6w10°C}$) exhibited the smallest change in the dissolution profile in relation to the dissolution profile of pellets after coating. In conclusion, storage conditions had an insignificant impact on the release rate of the model drug in phosphate buffer.

3.8.7. Differential Scanning Calorimetry analyses of pellets stored under different temperature and humidity conditions

Storage at 33 % RH (21°C)

During the first heating a melting peak at 59.2°C appeared. Over cooling two solidification points at 48.8°C and at 43.3°C were detected. The second heating revealed three melting peaks, at 46.7°C, at 51.3°C and at 58.1°C (Figure 85).

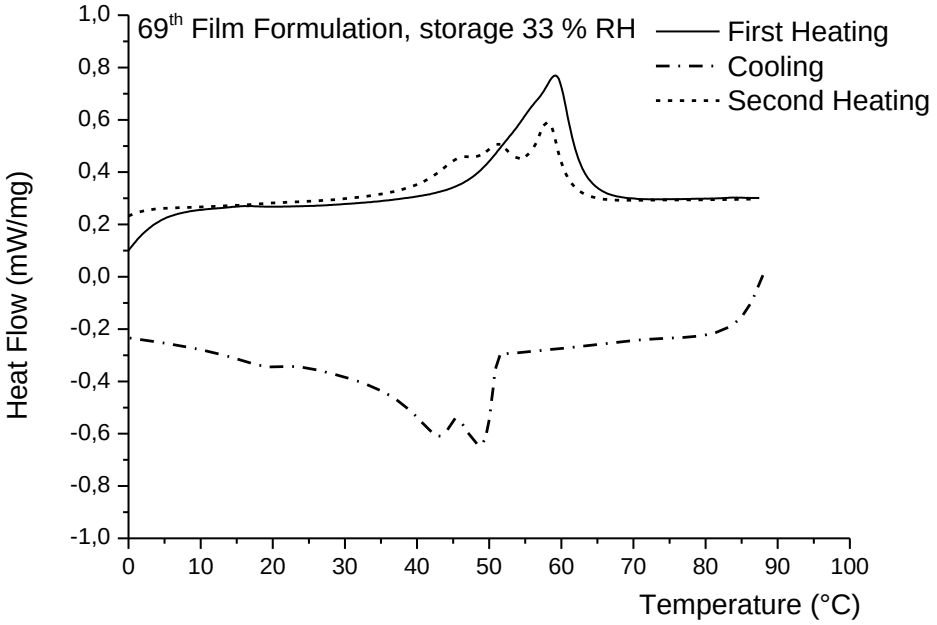


Figure 85. Differential Scanning Calorimetry thermogram of pellets coated with 69th formulation stored at 33 % RH, 21°C.

Storage at 76 % RH (21°C)

Over the first heating the whole sample melted at 59.4°C. During cooling two crystallization points at 49.8°C and at 42.8°C were observed. Two melting peaks were detected while the second heating of the sample, namely at 51.0°C and at 57.2°C (Figure 86).

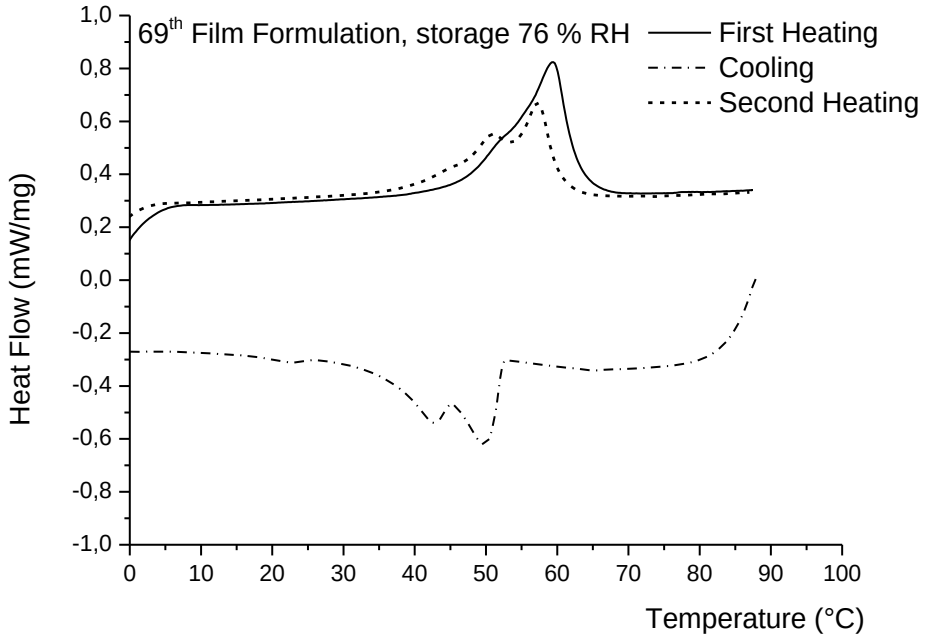


Figure 86. Differential Scanning Calorimetry thermogram of pellets coated with 69th formulation stored at 76 % RH, 21°C.

Storage at 10°C (fridge, no humidity-controlled storage)

Over the first heating a broad melting peak with the maximum at 58.3°C was detected. During temperature reduction of the sample two exothermic crystallization peaks at 49.6°C and at 42.7°C were noticed. After a renewed heating three melting peaks at 46.1°C, at 50.3°C and at 57.7°C were observed (Figure 87).

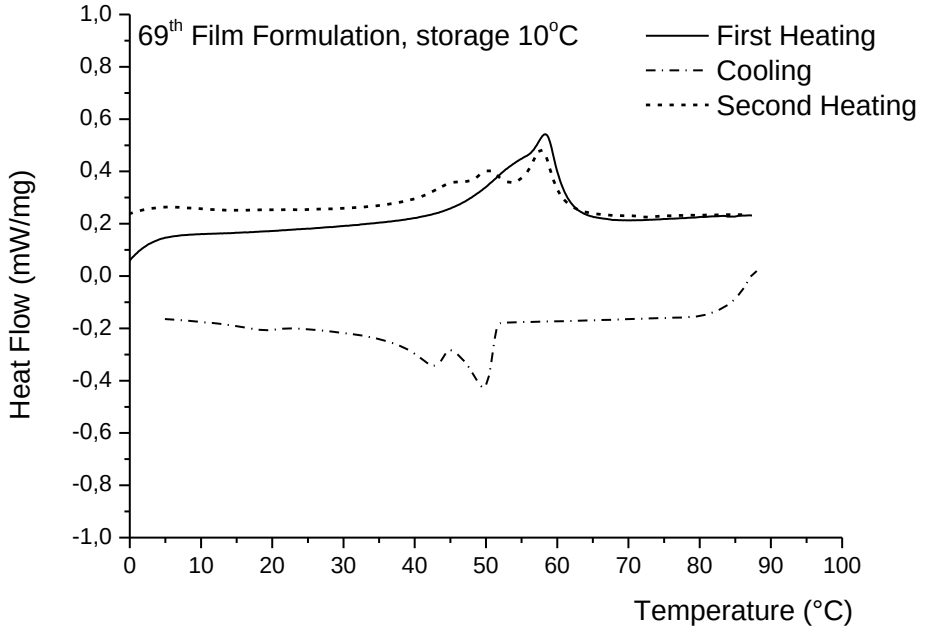


Figure 87. Differential Scanning Calorimetry thermogram of pellets coated with 69th formulation stored at 10°C.

3.8.8. Differential Scanning Calorimetry analyses of pellets stored under different temperature and humidity conditions-summary

The DSC curves of coated pellets stored under different conditions varied between each other. During the first heating the broadest melting peak was observed for the sample stored in the fridge (at 10°C). The melting peaks of samples stored at 33% RH and at 76% RH were narrower. For each formulation two crystallization points were noticed. After second heating for samples stored at 10°C and at 33 % RH three melting points were detected. However, in both cases melting peaks with the lowest temperature were barely visible. The results of DSC analyses indicated the influence of the storage conditions on the coating formulation.

3.8.9. Investigation of the influence of the film layer thickness on the dissolution kinetic of the model drug released from lipid coated pellets

MCC-based pellets prepared per extrusion/spheronization technique were coated with the 69th lipid formulation. The coating process of pellets was performed as described in Chapter 2.2.5. The thickness of the coating was 9.8 mg/cm^2 (F69''t_0). Afterwards the coated pellets were subjected to dissolution tests. The release profiles were next compared with the release profiles of pellets with a coating thickness of 13.9 mg/cm^2 (F69't_0, Figure 73 (pH 1.2) and Figure 74 (pH 6.8)). Dissolution profiles of pellets with a coating thickness of 13.9 mg/cm^2 were already presented in Chapter 3.8.4.

Dissolution tests of pellets with a coating thickness of 9.8 mg/cm^2

After 1 hour the amount of released caffeine in HCl medium (pH 1.2) had a value of 1.3 %. After the following hour the amount increased to 4.7 %. Over 3 hours of the test 11.6 % of the active substance were released (Figure 88).

Over the first hour of the dissolution test 51.2 % of the model drug were released in phosphate buffer (pH 6.8). After 2 hours already 92.7 % were detected. After the following hour almost all quantity of caffeine was released, namely 98.2 % (Figure 89).

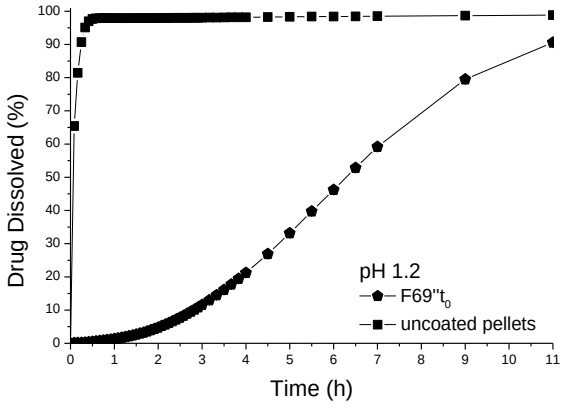


Figure 88. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th film formulation (F69''t_0, 9.8 mg/cm2) in hydrochloric acid medium (pH 1.2).

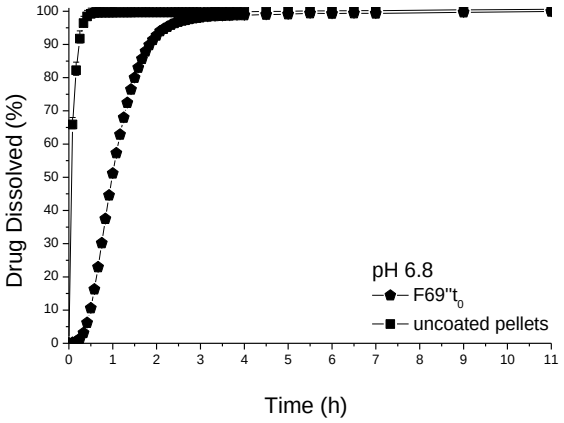


Figure 89. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th film formulation (F69''t_0, 9.8 mg/cm^2) in phosphate buffer (pH 6.8).

Dissolution tests of pellets coated with a film thickness of 13.9 mg/cm^2 (F69't_0) and of 9.8 mg/cm^2 (F69''t_0)-comparison

In HCl medium pellets covered with a thinner film layer (F69''t_0, 9.8 mg/cm^2) showed a faster release of the model drug in comparison to pellets coated with a thicker film layer (F69't_0, 13.9 mg/cm^2, Figure 73). For the first 30 minutes the differences in the release rate were only slight. Subsequently, the distinctions were gradually growing.

Also in phosphate buffer pellets coated with a thinner film layer (F69''t_0, 9.8 mg/cm^2) released the active substance faster than pellets covered with a thicker film layer (F69't_0, 13.9 mg/cm^2, Figure 74).

Analyzing above presented results of the dissolution tests it could be assumed that due to an adequate layer thickness a desired release delay of an active substance from pellets coated with developed lipid coating could be obtained.

3.8.10. Influence of storage conditions on the release rate of caffeine from pellets with a coating thickness of 9.8 mg/cm^2

Pellets with a coating thickness of 9.8 mg/cm^2 were stored in a fridge (at 10°C) for 10.5 weeks (F69''$t_{10.5w10°C}$) and then subjected to dissolution tests. Thereafter, obtained dissolution profiles were compared with dissolution profiles of pellets analyzed directly after coating (F69''t_0, Figure 88 (pH 1.2) and Figure 89 (pH 6.8)).

The quantity of released caffeine in hydrochloric acid medium (pH 1.2) from stored pellets (F69''$t_{10.5w10°C}$) after the first hour was 1.8 %. After 2 hours the amount increased to 6.0 %. Over 3 hours 14.3 % of the API were detected (Figure 90).

The differences in the release rates between pellets analyzed after coating (F69''t_0, Figure 88) and pellets stored for 10.5 weeks in a fridge (F69''$t_{10.5w10°C}$) for the first 2 hours in HCl medium were very small. Afterwards, the variations were steadily growing. From gastro-resistant dosage form's point of view, the differences in the release rates of F69''t_0 and F69''$t_{10.5w10°C}$ occurred after 2 hours of pellets incubation in HCl medium can be ignored.

In phosphate buffer (pH 6.8) the quantity of released caffeine from stored pellets (F69''$t_{10.5w10°C}$) after 1 hour amounted to 57.0 %. After 2 hours 95.1 % were already detected. Within 3 hours the release of the model drug was almost complete and reached 98.4 % (Figure 91).

The dissolution profiles in phosphate buffer of pellets analyzed after their coating (F69''t_0, Figure 89) and after their storage for 10.5 weeks at 10°C (F69''$t_{10.5w10°C}$) showed very similar course. Only little higher release rate of F69''$t_{10.5w10°C}$ compared to F69''t_0 was observed.

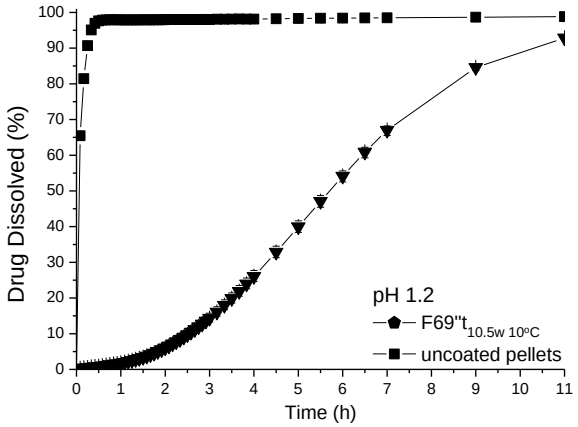


Figure 90. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th film formulation (F69''t_0, 9.8 mg/cm^2) in hydrochloric acid medium (pH 1.2).

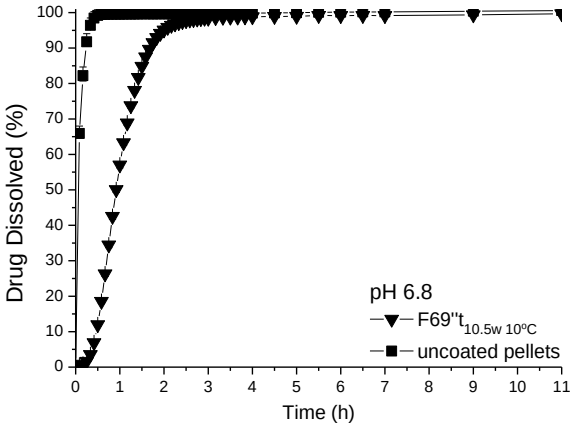


Figure 91. Dissolution kinetic of caffeine released from uncoated pellets and from pellets coated with 69th film formulation (F69''t_0, 9.8 mg/cm^2) in phosphate buffer (pH 6.8).

The above presented results pointed at an acceptable stability of the coating formulation during pellets storage at 10°C (in the fridge) during the tested period.

4. Summary and perspectives

The aim of the present research was to develop innovative enteric coatings. The composition of these coatings should include only edible and biodegradable substances which are generally recognized as safe (GRAS). The main components of the coating formulations were lipids. Their presence in the coating material had to determine the release of the active substance. Lipids, due to their behavior in the gastrointestinal tract, could potentially form coatings assuring an effective enteric drug delivery. Because of lipids' natural origin, they can be a favorable coating material in food products or nutritional supplements.

First a careful screening of potential lipids of coating formulations was carried out. It was examined how the substances behave in different dissolution media. Their stability in an acidic environment for at least two hours was required. After a physiologically relevant change of the dissolution medium to a higher pH value (small intestine pH) the drug release should take place. The increase of lipids amount in the film resulted in greater water resistance. The modification of water resistance by using different lipids at different concentrations allowed to control the duration of the lag phase. The rational development of lipid-based enteric coatings has to consider the biofate of the film, which includes the possible interaction with mixed micelles and enzyme induced lipid digestion. The main digestion of lipids takes place in the small intestine. Therefore, a film with an appropriate amount of lipids will exhibit a resistance to gastric juice. Following the investigation of lipids it was decided which lipids could be advantageous in the coating mixture. The key parameters here were the melting temperatures of lipids and their behavior in dissolution media (hydrochloric acid buffer pH 1.2 and phosphate buffer pH 6.8). The melting temperatures play a very important role in the production of coating dispersions and in the coating processes. The behavior at different pH values already gave the first indications of the behavior in the gastrointestinal tract. Unfortunately, lipids are usually very poor film formers. Films made from lipids only are very brittle and inflexible. To overcome the brittleness and to achieve the desired flexibility, film-forming agents and plasticizers were added to the coating formulations. Moreover, the use of emulsifiers and stabilizers enabled the creation of stable coating dispersions. After all the potential excipients of the enteric coating formulations were chosen, coating dispersions were prepared.

In the current work the manufacturing technology of coating dispersions was developed and optimized. The most important factors of the production technology are the process temperature, order of component addition and stirring time. The aim was to develop a possibly simple, inexpensive and reproducible method. A prepared coating dispersion should possess adequate properties (stability, homogeneity, proper viscosity, sprayability) and should also form a film with proper attributes (sufficient mechanical strength, flexibility, homogeneity, adequate water resistance

and/or stability for 2 hours in aqueous solutions with low pH values). Only dispersions with good quality (appropriate particle size, low viscosity, sufficient stability) were used for film formation. In the first screening test, the dispersions were cast on a PTFE surface and the obtained film was evaluated. If the film showed good characteristic, the dispersion was then sprayed on a PTFE surface and the formed film was further investigated. Film evaluation included: disintegration tests, pH-dependency and water resistance tests in several dissolution media (HCl medium, phosphate buffer, Fed State Simulated Intestinal Fluid (FeSSIF) with and without an addition of pancreatin). A number of dispersions and films were created and investigated. Many modifications of compositions were necessary to obtain both a good dispersion and an appropriate film. After many attempts, a formulation (48th formulation) showing adequate properties was found. This coating dispersion was stable, homogeneous and readily sprayable, which played an important role in the subsequent coating process of pellets. The sprayed film was homogeneous, flexible and possessed good mechanical strength. The disintegration of this film during incubation in dissolution media could not be observed after several hours (disintegration test). It also constituted a very good water barrier (water resistance test). Next, the ability of the 48th formulation for the film formation on pellets surface was tested. For this purpose, placebo MCC-pellets were taken. The coating of pellets was carried out using the fluidized bed process. Unfortunately, the 48th dispersion was not able to form a film on pellets' surface. The dry ingredients of the formulation were deposited in the device and on the filter. For this reason, further modifications of the coating dispersion were performed. At the same time, the coating parameters were optimized. After the composition of the dispersion was modified, further coatings experiments were carried out. The following aspects were simultaneously evaluated: the formation of the coating on pellets surface, the agglomeration of pellets, and the influence of coating parameters on the film formation. Depending on the needs appropriate excipients were replaced or their concentration was changed. The parameters were adjusted accordingly for the proper coalescence of particles on the pellets' surface and for the avoidance of pellets' agglomeration. A major challenge was to develop a coating dispersion possessing good film-forming abilities and simultaneously forming a film with sufficient resistance to water and pH-sensitivity. In the coating formulations only water-soluble film formers were used in order to avoid organic solvents. A high proportion of water-soluble polymers led to better film-forming abilities, but also to but also to a decreased water resistance of the coating. Only adequate resistance to water could however ensure a delayed release of the active ingredient (lag phase).

18 further coating dispersions were prepared and evaluated. Finally, a dispersion was developed (the 66th formulation), which formed a film with desirable properties. During the coating process no agglomerates were formed. The film demonstrated good mechanical strength and was sufficiently flexible. The coating also exhibited pH-sensitivity. The investigations of the

66th formulation included: the assessment of sprayed film (water resistance test in HCl medium and in simulated intestinal fluid) and the evaluation of coated pellets (pH-dependency test, film permeability test, EPR studies and dissolution tests). The 66th coating exhibited very good water resistance. The sprayed film was impermeable to methylene blue in the simulated gastric juice. After 2 hours of incubation, the medium remained colorless. The color of the simulated intestinal fluid (pH 6.8, containing digestive enzymes and bile salts) changed only after 5.5 hours, indicating the film permeability. The coating on MCC-placebo pellets also showed a pH-dependent behavior. After 2 hours' incubation in HCl medium no visible changes of the coating could be observed, and the film remained hard. In contrast, a swelling of the coated film was observed after incubation in phosphate buffer. The film became very soft and easy to remove. The first idea about the release modification by the coating provided the film permeability test of coated methylene blue-MCC pellets. These pellets were first incubated in hydrochloric acid medium (pH 1.2) and in phosphate buffer medium (pH 6.8). This experiment demonstrated that the 66th formulation ensures a modified release from pellets. The color saturation of the dissolution media increased during the test progress. In addition, the release in pH 6.8-medium was faster than in pH 1.2-medium (pH-dependency). Moreover, the release occurred in the acidic medium with 3 hours' delay (lag phase). Since lipids are digested in gastrointestinal tract, the film permeability test was also performed in simulated intestinal fluids (FeSSIF). Parallel tests in the medium containing only mixed micelles and in the medium containing mixed micelles and pancreatin were carried out. At first, pellets were incubated in HCl medium for 2 hours. Thereafter, the medium was changed to simulated intestinal fluid (FeSSIF). The change of the medium color without the addition of pancreatin occurred one hour later than the change of the medium color containing bile salts, phospholipids and pancreatin. This experiment indicated that the presence of pancreatin accelerated the drug release. The lipids of the coating are hydrolyzed by digestive enzymes, thus the permeability of the film increased. Subsequently, the penetration of water through the film was examined. The test was conducted using Electron Spin Resonance (ESR) spectroscopy. The experiment revealed the pH-sensitivity of the film. Water penetrated faster through the coat in phosphate buffer than in acidic medium. This testified that the release of the drug will be quicker in the small intestine than in the stomach. The pH-dependent behavior of the developed coating was further confirmed in the dissolution tests. For these tests pellets with model drug caffeine were prepared via saturation technique. The caffeine pellets were next coated with the 66th formulation. The dissolution tests were carried out in the simulated gastric juice (hydrochloric acid buffer, pH 1.2) and in the artificial small intestine fluid (phosphate buffer, pH 6.8). Only about 4.5 % of the active ingredient was released after 2 hours' incubation in the simulated gastric fluid. In contrast, about 82.6 % of API was released in the phosphate buffer medium.

Next, additional modifications of the 66th formulation were performed. The 66th coating formulation was a good starting point for the development of further lipid-based coatings. Pellets coated with this coating showed an adequate modification of the drug release profile. The aim of the additional alterations of the 66th formulation was to obtain a lipid coating with a longer lag phase with close to zero API release in the hydrochloric acid medium (pH 1.2), and simultaneously a fast release at higher pH values (phosphate buffer, pH 6.8). As a result three additional coatings (67th, 68th and 69th formulation) were obtained which showed the desired delayed, pH- and time-dependent release of the active substance. The longest lag-phase was observed for pellets coated with the 69th coating dispersion, whereas the fastest release demonstrated the 67th formulation. Thereafter, pellets coated with the developed formulations were stored at room temperature for 10.5 weeks and subjected to dissolution tests. The release rates of stored pellets ($Ft_{10.5w}$) were higher than release rates obtained from pellets analyzed right after their coating (Ft_0). However, during 2 hours' incubation in HCl medium the release profiles of all stored pellets did not differ much from release profiles of Ft_0 pellets. Subsequently, the differences were increasing. The dissolution profiles in phosphate buffer differed only slightly between Ft_0 and $Ft_{10.5w}$ during the whole test period. After the storage the longest delay in the drug release was still demonstrated by the 69th formulation and the shortest by the 67th formulation. As the 69th coating dispersion showed very good properties and pellets coated with this formulation exhibited the highest stability, it was taken for further examinations.

In the next part of this work the 69th formulation was subjected to more detailed analysis. For these studies pellets with a defined content of a model drug were prepared. These pellets, containing caffeine as a model drug, were manufactured via extrusion/spheronization method and coated with the lipid-based formulation (the layer thickness of the coated pellets was 13.9 mg/cm^2). Thereafter, dissolution tests in HCl and in phosphate media were conducted. In hydrochloric acid medium after 2 hours of pellets incubation only about 1.5 % of the active ingredient was released. In contrast, in phosphate-medium 73.9 % of the API was released. Afterwards, the coated pellets were stored under various conditions (33 % RH (21°C), 76 % RH (21°C), 10°C- in a fridge) and for different period of time (two and six weeks). Following the evaluation of the test, an influence of storage conditions on the release characteristics was found. The highest release rate was observed for pellets stored at 76% RH. The release profiles of pellets stored at 33 % RH up to 2 hours differed only slightly from the release profiles of pellets analyzed directly after their coating ($F69t_0$). After pellets storage in the refrigerator (at 10°C) basically no differences in the drug release in comparison to the dissolution profiles of $F69t_0$ pellets could be noticed. Also after 2 hours the profiles showed very similar course. In the next study the influence of the film thickness on the release rate of the model drug was investigated. Pellets with a thinner film (9.8 mg/cm^2) released

caffeine faster, compared to pellets coated with a thicker film (13.9 mg/cm^2). Then pellets with the layer thickness of 9.8 mg/cm^2 were stored for 10.5 weeks in a fridge (10°C) and subjected to dissolution tests. Here, the influence of storage conditions on drug release could also be observed. The release of the active ingredient was faster for stored pellets than for F69t_0 pellets. The differences in the release rate in HCl medium were, however, insignificant for the first 2 hours.

After very time-consuming searching of components and screening of film formulations, four lipid-based coatings were developed. Every formulation demonstrated a delayed, pH- and time-dependent release characteristic. Therefore, each of these coatings could be used in dosage forms, where delayed release is desired. Furthermore, three of four developed coatings could be potentially used in gastro-resistance dosage forms. Additionally, the composition of the developed coatings is very simple, all ingredients are edible, biodegradable and commonly used as food additives. The fundamental substances of these formulations represent lipids. Due to their presence in the coating material a delayed and pH-dependent release of the active substance was achieved. Further, the simple preparation of the coating dispersions is very favorable from the economic point of view. This work constitutes an important starting point for further research on the developed lipid-based coating formulations. It is crucial to continue the existing research. Further work should include: film permeability tests in simulated gastric and intestinal fluids, further stability tests, the investigation of the release mechanism, the study of the cause of the changes in the dissolution kinetic after storage under different conditions, etc.

The developed lipid-based coatings can be an alternative for shellac coatings used in enteric drug delivery systems. Shellac shows good resistance in gastric fluids, but a considerable problem is the slow dissolution of shellac coatings in higher pH media, such as intestinal fluids [22]. The developed lipid coatings provide a good barrier in gastric environment and their permeability significantly increase in higher pH value (intestinal milieu). So far, no effective pH-sensitive coating was developed that employ nontoxic, biodegradable substances and simultaneously ensure a reliable enteric drug delivery. Therefore, the developed lipid-based enteric coatings are a major achievement and constitute a great progress in the development of enteric formulations.

5. Zusammenfassung und Ausblick

Ziel der Forschung war es, neue und innovative magensaftresistente Überzüge zu entwickeln. Diese Überzüge sollten nur essbare und biologisch abbaubare Stoffe enthalten, die einen GRAS-Status besitzen (Generally Recognized As Safe). Als Hauptkomponenten der Überzugsformulierungen wurden Lipide ausgewählt. Ihre Anwesenheit im Überzugsmaterial sollte die Freisetzung des Wirkstoffs bestimmen. Aufgrund ihres Verhaltens im Gastrointestinaltrakt können Lipide potentiell Überzüge bilden, die eine effektive, enterale Arzneimittelabgabe gewährleisten. Da Lipide natürlicher Herkunft sind, können sie ein wertvolles Überzugsmaterial sein, das in Lebensmitteln oder Nahrungsergänzungsmittel eine Anwendung findet.

Am Anfang der Arbeit wurde ein sorgfältiges Screening von potenziellen Lipiden durchgeführt. Es wurde geprüft, wie sich die Substanzen in Freisetzungsmedien verhalten. Erwünscht war die Stabilität der Substanzen in Säuren und/oder die Stabilität der Substanzen bis zwei Stunden nach der Einnahme (Lag-Phase). Nach der physiologisch relevanten Änderung des Freisetzungsmediums zu höheren pH-Werten (Dünndarm-pH) sollte die Wirkstofffreisetzung stattfinden. Die funktionellen Grundsubstanzen der magensaftresistenten Formulierungen stellten Lipide dar. Durch ihre Anwesenheit in dem Überzugsmaterial sollte eine pH-abhängige Wirkstofffreisetzung erreicht werden. Eine Erhöhung ihres Anteils führte zu einer größeren Wasserfestigkeit. Die Modifizierung der Wasserfestigkeit durch Einsatz verschiedener Lipide in verschiedenen Konzentrationen ermöglichte eine gezielte Beeinflussung der Dauer der Lag-Phase. Bei der Entwicklung der Lipidüberzüge war auch zu beachten, dass Lipide im Körper durch Verdauungsenzyme abgebaut werden und mit Mischmizellen wechselwirken können (z.B. Monoglyceride und Fettsäuren). Der Hauptverdau von Lipiden findet im Dünndarm statt. Deswegen kann ein Film mit einer entsprechenden Lipidmenge eine Magensaftresistenz aufweisen. Es wurden durch mich Eigenschaften von einigen potenziell geeigneten Lipiden geprüft. Danach wurde entschieden, welche Lipide in der Mischung vorteilhaft sein könnten. Wichtige Parameter waren hierbei die Schmelztemperaturen der Lipide und ihr Verhalten in verschiedenen Medien (Salzsäure-Puffer pH 1.2, Phosphatpuffer pH 6.8). Die Schmelztemperaturen der Lipide spielten für die Herstellungstechnologie der Coatingdispersionen und für den Coatingprozess eine sehr wichtige Rolle. Das Verhalten bei unterschiedlichen pH-Werten ergab bereits erste Hinweise auf das Verhalten im Magen-Darm-Trakt. Außer Lipiden war eine Zugabe weiterer Komponenten erforderlich, um ein gutes Überzugsmaterial zu bilden. Lipide sind meist sehr schlechte Filmbildner. Reine Lipidfilme sind sehr spröde und wenig flexibel. Um ein entsprechendes Überzugsmaterial zu erhalten, musste die Mischung neben Lipiden auch andere Substanzen enthalten (filmbildende Polymere, Emulgatoren und Weichmacher). Die Substanzen waren notwendig, damit das Material passende Eigenschaften

aufwies. Nachdem alle potenziellen Hilfsstoffe gewählt wurden, wurden Coatingdispersionen hergestellt.

In der vorliegenden Arbeit wurde die Herstellungstechnologie der Überzugsdispersionen entwickelt und optimiert. Die wichtigsten Faktoren der Herstellungstechnologie sind die Prozesstemperatur, die Reihenfolge der Zugabe der Bestandteile und die Rührzeit. Das Ziel war ein möglichst einfaches, preiswertes und reproduzierbares Verfahren zu entwickeln. Eine hergestellte Coatingdispersion sollte angemessene Eigenschaften besitzen (Stabilität, Homogenität, richtige Viskosität, Sprühbarkeit) und sollte außerdem einen guten Film bilden (ausreichende mechanische Festigkeit, Flexibilität, Homogenität, genügende Wasserfestigkeit und Stabilität während 2 Stunden in Säuren). Die Optimierung der Dispersionen erfolgte in einem mehrstufigen Verfahren. Zuerst wurde sie auf einer Polytetrafluoroethylene (PTFE)-Oberfläche gegossen, und der erhaltene Film wurde bewertet. Wenn der Film eine gute Charakteristik zeigte, wurde dann die Dispersion auf einer PTFE-Oberfläche gesprüht, und der gebildete Film wurde weiter untersucht. Die Filmauswertung umfasste Zerfalls-, pH-Abhängigkeits- und Wasserfestigkeitstests in mehreren Freisetzungsmedien (HCl-und Phosphatpuffer-Medium, simulierte Darmflüssigkeit (FeSSIF) mit und ohne Zusatz von Verdauungsenzymen). Eine Reihe von Dispersionen und Filmen wurden hergestellt und untersucht. Viele Modifikationen der Zusammensetzungen waren erforderlich, um sowohl eine gute Dispersion als auch einen geeigneten Film zu erhalten. Nach vielen Versuchen wurde schließlich eine Formulierung (die 48. Formulierung) hergestellt, welche die gewünschten Eigenschaften besaß. Diese Coatingdispersion war stabil, homogen und gut sprühbar, was für die Beschichtung der Pellets eine wichtige Rolle spielte. Der gesprühte Überzug war homogen, wenig spröde und besaß eine gute mechanische Festigkeit. Der Zerfall des Films in Freisetzungsmedien konnte auch nach vielen Stunden nicht beobachtet werden (Zerfalltest). Er stellte auch eine sehr gute Wasserbarriere dar (Wasserfestigkeitstest). Als Nächstes wurde die Fähigkeit der 48. Überzugsformulierung zur Bildung eines Filmes auf der Oberfläche von Pellets geprüft. Zum Überziehen wurden Placebo-Pellets ausgewählt. Das Überziehen von den Pellets wurde anhand des Wirbelschichtverfahrens durchgeführt. Leider bildete die 48. Dispersion keinen Überzug auf den Pellets. Die trockenen Bestandteile der Formulierung haben sich in dem Gerät und auf dem Filter abgelagert. Aus diesem Grund wurden weitere Modifikationen der Coatingdispersion durchgeführt. Dabei wurden Coatingparameter optimiert. Nach der Änderung der Zusammensetzung der Dispersion wurden weitere Coatingversuche durchgeführt. Dabei erfolgte eine Beurteilung folgender Aspekte: die Entstehung des Überzugs auf den Pellets, die Agglomeration der Pellets, der Einfluss der Coatingparameter auf der Filmbildung. Zur Optimierung wurden Hilfsstoffe ausgetauscht oder ihre Konzentration geändert. Die Parameter wurden entsprechend für die korrekte Koaleszenz der Partikel auf der Pelletoberfläche und für die Vermeidung der Agglomeration angepasst. Eine sehr

große Herausforderung war eine Dispersion zu entwickeln, die einen Film mit einer genügenden Resistenz gegen Wasser bildete und gleichzeitig gute Filmbildungsfähigkeiten besaß. Als Filmbildner in den Formulierungen wurden nur wasserlösliche Filmbildner benutzt, um organische Lösungsmittel zu vermieden. Ein hoher Anteil wasserlöslicher Polymere führte zu besseren Filmbildungsfähigkeiten, aber auch zur geringeren Wasserfestigkeit des Überzuges. Eine ausreichende Resistenz gegenüber Wasser ist für das erwünschte Freisetzungsprofil notwendig.

Es wurden weitere 18 Coatingdispersionen hergestellt und beurteilt. Schließlich wurde eine Dispersion erfunden (66. Formulierung), die einen Film mit den geforderten Eigenschaften bildete. Während des Überziehens sind keine Agglomerate entstanden. Der Film besaß eine gute mechanische Festigkeit und war hinreichend flexibel. Der Überzug wies auch ein pH-abhängiges Verhalten auf. Die Untersuchungen der 66. Formulierung umfassten: die Beurteilung des gesprühten Films (Wasserfestigkeitsprüfung im HCl-Medium und in der simulierten Darmflüssigkeit) und die Bewertung der beschichteten Pellets (pH-Abhängigkeitstests, Filmdurchlässigkeitstests, EPR-Studien und Wirkstofffreisetzungstests). Der 66. Coating zeigte eine sehr gute Wasserresistenz. Der gesprühte Film war undurchlässig für Methylenblau im künstlichen Magensaft. Nach der 2 stündigen Inkubation ist das Medium farblos geblieben. Die Farbe der simulierten Darmflüssigkeit (pH 6.8, enthält Verdauungsenzyme und Gallensalze) hat sich erst nach ca. 5,5 Stunden geändert, was auf Filmdurchlässigkeit hindeutete. Der Überzug auf den Placebo-MCC-Pellets zeigte auch ein pH-abhängiges Verhalten. Nach zweistündiger Inkubation im HCl-Medium wurden keine sichtbaren Veränderungen der Beschichtung festgestellt. Der Film blieb hart und mechanisch stabil. Im Gegensatz dazu sah der Überzug nach der Inkubation im Phosphatpuffer-Medium geschwollen aus. Er war sehr weich und leicht zu entfernen. Die ersten Daten über die Modifizierung der Freisetzung durch den Filmüberzug lieferte der Filmdurchlässigkeitstest der überzogenen Methylenblau-MCC-Pellets. Die Pellets wurden zuerst im künstlichen Magensaft (HCl-Medium, pH-Wert 1.2) und im künstlichen, enzymfreien Dünndarmmedium (Phosphatpuffer, pH 6.8) inkubiert. Die Versuche zeigten, dass die 66. Formulierung eine modifizierte Freisetzung aus den Pellets sichert. Die Farbsättigung der Medien stieg im Laufe des Tests. Darüber hinaus war die Freisetzung im pH 6.8 Medium schneller als im pH 1.2-Medium (pH-Abhängigkeit). Die Freisetzung im künstlichen Magensaft zeigte eine Verzögerung (Lag-Phase) von ca. 3 Stunden. Da Lipide im Magen-Darm-Trakt verdaut werden, wurde der Filmdurchlässigkeitstest auch in der simulierten Darmflüssigkeit geprüft. Parallel wurden Versuche im Medium, das nur Mischmizellen enthielt, und im Medium, das Mischmizellen und Pankreatin enthielt, durchgeführt. Zuerst wurden die überzogenen Pellets 2 Stunden im künstlichen Magensaft (HCl-Medium, pH-Wert 1.2) inkubiert. Danach wurde das Medium vom künstlichen Magensaft auf die simulierte Dünndarmflüssigkeit (pH 6.8) gewechselt. Die Änderung der Farbe des Mediums ohne die Zugabe von Pankreatin trat eine Stunde später als die

Änderung der Farbe des Mediums mit Gallensalzen, Phospholipiden und Pankreatin auf. Der Versuch zeigte, dass Pankreatin die Freisetzung beschleunigt. Die Lipide des Überzugs wurden verdaut, dadurch stieg die Durchlässigkeit des Films. Anschließend wurde die Permeation des Wassers durch den Film untersucht. Die Prüfung wurde anhand der ESR-Methode (Elektronenspinresonanz) durchgeführt. Der Versuch hat auch die pH-abhängigen Eigenschaften des Films bewiesen. Das Wasser drang schneller durch den Film im künstlichen Dünndarmmedium (pH 6.8) als im künstlichen Magensaft. Das bezeugte, dass die Freisetzung des Wirkstoffes im Dünndarm schneller als im Magen sein wird. Die pH-abhängige Freisetzung wurde weiter in den Freisetzungstests bestätigt. Für die Freisetzungstests wurden Pellets mit dem Modellwirkstoff Coffein durch Sättigungstechnik hergestellt. Die Coffeinpellets wurden mit der 66. Formulierung überzogen. Der Freisetzungstest wurde im künstlichen Magensaft (Salzsäure-Puffer, pH-Wert 1.2) und in der simulierten Dünndarmflüssigkeit (Phosphatpuffer, pH 6.8) vorgenommen. Im künstlichen Magensaft wurden nach 2 Stunden nur ca. 4.5 % des Wirkstoffes freigesetzt. Im Unterschied dazu wurden in der künstlichen Dünndarmflüssigkeit ca. 82.6 % freigesetzt.

Als Nächstes wurden zusätzliche Modifikationen der 66. Coatingdispersion durchgeführt. Die 66. Coatingformulierung war ein guter Ausgangspunkt für die Entwicklung weiterer Lipidüberzüge. Ziel der zusätzlichen Änderungen war, ein Lipidcoating zu erhalten, die eine längere Verzögerungsphase mit minimaler API-Freisetzung im Salzsäuremedium (pH 1,2) und gleichzeitig eine schnelle Freisetzung bei höheren pH-Werten (Phosphatpuffer, pH 6,8) aufweist. So wurden drei weitere Formulierungen (67., 68. und 69.) geschaffen, die eine verzögerte, pH- und zeitabhängige Freisetzung des Wirkstoffs aufwiesen. Die längste Lag-phase wurde bei den Pellets beobachtet, welche mit der 69. Dispersion überzogen wurden. Die schnellste Freisetzung des Wirkstoffes zeigte die 67. Formulierung. Überzogene Pellets mit den entwickelten Coatingdispersionen wurden danach 10.5 Wochen bei Raumtemperatur gelagert und Freisetzungstests unterzogen. Die Freisetzungsraten von gelagerten Pellets ($Ft_{10.5w}$) waren höher als die Freisetzungsraten von Pellets, die direkt nach dem Überziehen analysiert wurden (Ft_0). Während zweistündiger Inkubation im HCl-Medium unterschieden sich die Freisetzungsprofile aller gelagerten Pellets jedoch gering von Freisetzungsprofilen der Ft_0 Pellets. Die Freisetzungsprofile im Phosphatpuffer-Medium unterschieden sich unbedeutend zwischen Ft_0 und $Ft_{10.5w}$ während der gesamten Testperiode. Nach der Lagerung zeigte die 69. Formulierung die längste Verzögerung der Wirkstofffreisetzung und die 67. Formulierung die kürzeste. Da die 69. Coatingdispersion sehr gute Eigenschaften aufwies und Pellets überzogen mit dieser Dispersion die höchste Stabilität aufzeigten, wurde sie genauer untersucht.

Im nächsten Teil dieser Arbeit wurde die 69. Formulierung einer genaueren Analyse unterzogen. Für diesen Zweck wurden Pellets mit einem definierten Gehalt eines Modellwirkstoffes

hergestellt. Als Modellwirkstoff wurde Coffein verwendet. Diese Pellets wurden durch Extrusion/Sphäronisation produziert und mit der 69. Coatingdispersion überzogen (Schichtdicke 13.9 mg/cm^2). Danach wurde der Freisetzungstest im HCl-Medium und im Phosphatpuffer vorgenommen. Im Salzsäure-Puffer wurden nach 2 Stunden nur ca. 1.5 % des Wirkstoffes freigesetzt. Im Unterschied dazu wurden in der künstlichen Dünndarmflüssigkeit ca. 73.9 % freigesetzt. Anschließend wurden die überzogenen Pellets unter verschiedenen Bedingungen (33% RH (21°C), 76% RH (21°C), 10°C- im Kühlschrank) und für verschiedene Zeitperiode (zwei und sechs Wochen) gelagert. Nach der Bewertung des Tests wurde ein Einfluss der Lagerbedingungen auf die Freisetzungscharakteristik festgestellt. Die höchsten Freisetzungsraten wurden für Pellets beobachtet, die bei 76 % RH gelagert wurden. Die Freisetzungsprofile für Pellets, die bei 33 % RH gelagert wurden, unterschieden sich bis zu 2 Stunden nur gering von den Freisetzungsprofilen der Pellets, die direkt nach dem Überziehen analysiert wurden (F69't_0). Nach der Lagerung der Pellets im Kühlschrank (bei 10°C) konnten praktisch keine Unterschiede in der Wirktofffreisetzung im Vergleich zu den Freisetzungsprofilen der F69't_0 Pellets festgestellt werden. Auch nach 2 Stunden zeigten die Freisetzungsprofile einen sehr ähnlichen Verlauf. In der nächsten Studie wurde der Einfluss der Filmschichtdicke auf die Freisetzungsrate des Modellwirkstoffes untersucht. Pellets mit einer dünneren Filmschicht (9.8 mg/cm^2) setzten Koffein schneller frei, im Vergleich mit Pellets mit einer dickeren Filmschicht (13.9 mg/cm^2). Danach wurden Pellets mit der Schichtdicke 9.8 mg/cm^2 für 10.5 Wochen in einem Kühlschrank aufbewahrt und Freisetzungstests unterzogen. Auch hier wurde der Einfluss der Lagerungsbedingungen auf die Wirkstofffreisetzung bemerkt. Die Freisetzung des Wirkstoffes war schneller für aufbewahrte Pellets als für F69't_0 Pellets. Die Unterschiede in der Freisetzungsrate waren jedoch für die ersten 2 Stunden unbedeutend.

Nach sehr zeitaufwendiger Suche von Komponenten und Screening von Filmformulierungen wurden vier Lipid-basierende Überzüge entwickelt. Jede Formulierung wies eine verzögerte, pH- und zeitabhängige Freisetzungscharakteristik des Wirkstoffes auf. Daher kann jede der entwickelten Formulierungen als ein Überzug für Arzneiformen verwendet werden, welche eine verzögerte Freisetzung erfordern. Zudem können drei von vier entwickelten Überzügen potenziell in magensaftresistenten Arzneiformen eingesetzt werden. Die Zusammensetzung der entwickelten Überzüge ist außerdem sehr einfach. Alle Bestandteile sind essbar, biologisch abbaubar und werden häufig als Lebensmittelzusatzstoffe verwendet. Die funktionellen Grundsubstanzen der Formulierungen stellen Lipide dar. Durch ihre Anwesenheit in dem Überzugsmaterial wurde eine verzögerte und pH-abhängige Wirkstofffreisetzung erreicht. Ferner ist die einfache Herstellung der Überzugsdispersionen aus wirtschaftlicher Sicht sehr günstig. Diese Arbeit stellt einen wesentlichen Ausgangspunkt für die weitere Forschung an den entwickelten Filmformulierungen

dar. Sie ist von großer Bedeutung, um die vorhandene Erforschung fortzusetzen. Die weitere Arbeit soll unter anderem umfassen: Coatingpermeabilitätstests in simulierten Magen- und Darmflüssigkeiten, weitere Stabilitätstests, die Untersuchung der Freisetzungsmechanismen, die Studie über die Ursache der Änderungen von Freisetzungsraten nach der Lagerung unter verschiedenen Bedingungen usw.

Die entwickelten Lipidüberzüge können eine Alternative für Schellacküberzüge sein, die in Darmwirkstofffreisetzungssystemen verwendet sind. Schellac zeigt eine gute Resistenz in der Magenflüssigkeit, aber ein großes Problem ist die langsame Auflösung von Schellacküberzügen in höheren pH-Medien, wie in der Darmflüssigkeit [22]. Die entwickelten Lipidüberzüge bieten eine gute Barriere in der Magen-Umgebung und ihre Permeabilität steigt deutlich in höheren pH-Werten (Darm-Milieu). Bisher wurde kein effektiver pH-empfindlicher Überzug entwickelt, der nicht toxische, biologisch abbaubare Substanzen einsetzt und gleichzeitig eine zuverlässige Darmwirkstoffgabe gewährleistet. Daher sind die entwickelten lipidbasierten Überzüge ein großer Erfolg und stellen einen großen Fortschritt in der Entwicklung von Darmformulierungen dar.

6. Appendix

6.1. References

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6.2. Table of abbreviations

AAP	Atomizing Air Pressure
AF	Air Flow
API	Active pharmaceutical ingredient
DSC	Differential scanning calorimetry
EPR	Electron paramagnetic resonance
ESR	Electron spin resonance
FeSSIF	Fed State Simulated Intestinal Fluid
GI	Gastrointestinal
GRAS	Generally Recognized As Safe
HCl	Hydrochloric acid
HPC	Hydroxypropyl cellulose
HPMC	Hydroxypropyl methylcellulose
IAT	Inlet Air Temperature
LCST	Lower Critical Solution Temperature
LPM	Liters per minute
MCC	Microcrystalline cellulose
MMC	Migrating myoelectric complex
MR	Modified-release
MUDF	Multiple-unit dosage forms
OAT	Outlet Air Temperature
OSA starch	Starch sodium octenyl succinate
Ph. Eur.	European Pharmacopoeia
PTFE	Polytetrafluoroethylene
RH	Relative humidity
rpm	Revolutions per minute
SR	Spray Rate
Tempol	4-Hydroxy-2,2,6,6,-tetramethypiperidin-1-oxyl (4 Hydroxy-TEMPO)
TGs	Triacylglycerols
UV	Ultraviolet

6.3. Conference Contributions

Dudzinska N, Hahn M, Mäder K. Development of MCC-free pellets with fast release for poorly soluble drugs. The 42nd Annual Meeting and Exposition of the Controlled Release Society, Edinburgh (Scotland) 2015. (Poster)

Dudzinska N, Hahn M, Mäder K. MCC-free pellets as an advantageous dosage form for poorly soluble drugs. 8th Polish-German Symposium on Pharmaceutical Sciences, Kiel (Germany) 2015. (Poster)

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6.5. Cirriculum Vitae

Persönliche Daten

Name	Natalia Magdalena Dudzińska
Geburtsdatum	18. Oktober 1988
Geburtsort	Działdowo/Polen

Ausbildung und Berufliche Entwicklung

10/2013-12/2016	Wissenschaftliche Mitarbeiterin am Institut für Pharmazie der Martin-Luther-Universität Halle Wittenberg
Seit 03/2014	Teilzeittätigkeit als angestellte Apothekerin in der Ahorn-Apotheke in Halle
10/2013	Approbation zum Apotheker (Deutschland)
06/2013-09/2013	Praktikum bei F. Hoffmann La-Roche, Abteilung Quality Assurance in Kaiseraugst, Schweiz
04/2013	Approbation zum Apotheker (Polen)
10/2012-03/2013	Pharmaziepraktikantin in der Brzezno-Apotheke in Danzig, Polen
03/2012-08/2012	Masterarbeit in der AG Pharmazeutische Technologie der Martin-Luther-Universität (Mag. Pharm.)
08/2011-09/2011	Pharmaziepraktikantin in der Krankenhausapotheke in Dzialdowo, Polen
06/2010-03/2013	Mitgliederin der International Society for Pharmaceutical Engineering (ISPE)
08/2010-09/2010	Pharmaziepraktikantin in der Brzezno-Apotheke in Danzig, Polen
10/2008-03/2012	Mitgliederin des wissenschaftlichen Kreises; Hauptaufgabe: Prüfung der antimikrobiellen Eigenschaften der Peptiden, Synthese der Peptide
10/2007-03/2013	Pharmaziestudium an der Medizinischen Universität zu Danzig, Polen
05/2007	Abitur an der Stefan Zeromski Gymnasium in Dzialdowo, Polen

_____ _____

Ort, Datum Natalia Dudzińska

6.6. Declaration of Original Authorship

Selbstständigkeitserklärung

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Arbeit selbstständig und ohne fremde Hilfe verfasst habe. Die Ergebnisse dieser Arbeit wurden unter Anleitung von Herrn Professor Dr. rer. nat. habil. Karsten Mäder selbstständig erarbeitet.

Weiterhin habe ich keine anderen als die angegebenen Quellen und Hilfsmittel verwendet und inhaltlich oder wörtlich übernommene Stellen als solche kenntlich gemacht. Beiträge von Kooperationspartnern zu den Ergebnissen dieser Arbeit habe ich eindeutig gekennzeichnet. Ich respektiere die Grundsätze guter wissenschaftlicher Praxis der DFG und habe keinerlei Daten gefälscht.

Ich habe bisher noch keine Promotionsversuche unternommen und diese Arbeit wurde keiner anderen Fakultät vorgelegt.

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Ort, Datum Natalia Dudzińska